☐1: AAC52479. FBP 17 [Mus muscu...[gi:1255033]

BLink, Nucleotide, OMIM, Related Sequences, PubMed, Taxonomy, LinkOut

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LOCUS       AAC52479                237 aa            linear   ROD 04-APR-1996
DEFINITION  FBP 17.
ACCESSION   AAC52479
PID         g1255033
VERSION     AAC52479.1  GI:1255033
DBSOURCE    locus MMU40751 accession U40751.1
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 237)
  AUTHORS   Chan,D.C., Bedford,M.T. and Leder,P.
  TITLE     Formin binding proteins bear WWP/WW domains that bind proline-rich
            peptides and functionally resemble SH3 domains
  JOURNAL   EMBO J. 15 (5), 1045-1054 (1996)
  MEDLINE   96183189
REFERENCE   2  (residues 1 to 237)
  AUTHORS   Chan,D.C., Bedford,M.T. and Leder,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-NOV-1995) David C. Chan, Genetics, Harvard Medical
            School, 200 Longwood Avenue, Boston, MA 02115, USA
COMMENT     Method: conceptual translation.
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                     /strain="FVB"
                     /db_xref="taxon:10090"
     Protein         1..237
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     CDS             1..237
                     /coded_by="U40751.1:<1..716"
                     /note="formin binding protein 17; contains SH3 domain"
ORIGIN
        1 kihcfrslkr ggvtpedfsn fppeqrrkkl qqkvddlnre iqketdqrda itkmkdvylk
       61 npqmgdpasl dqkltevtqn ieklrleaqk feawlaeveg rlparseqar rqsglydgqt
      121 hqtvtncaqd respdgsyte eqsqesehkv lapdfddefd deeplpaigt ckalytfegq
      181 negtisvveg etlsvieedk gdgwtrirrn edeegyfpts yvevyldkna kgaktyi
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Revised: October 24, 2001.

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LOGINID:SSSPTA1644PNH

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

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NEWS 3	Jan 29	FSTA has been reloaded and moves to weekly updates
NEWS 4	Feb 01	DKILIT now produced by FIZ Karlsruhe and has a new update frequency
NEWS 5	Feb 19	Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6	Mar 08	Gene Names now available in BIOSIS
NEWS 7	Mar 22	TOXLIT no longer available
NEWS 8	Mar 22	TRCTHERMO no longer available
NEWS 9	Mar 28	US Provisional Priorities searched with P in CA/CAplus and USPATFULL
NEWS 10	Mar 28	LIPINSKI/CALC added for property searching in REGISTRY
NEWS 11	Apr 02	PAPERCHEM no longer available on STN. Use PAPERCHEM2 instead.
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NEWS 16	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 17	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 18	Apr 22	Federal Research in Progress (FEDRIP) now available

NEWS EXPRESS	February 1 CURRENT WINDOWS VERSION IS V6.0d, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 05 FEBRUARY 2002
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NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
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* * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:00:29 ON 07 MAY 2002

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COST IN U.S. DOLLARS SINCE FILE TOTAL

	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 13:00:42 ON 07 MAY 2002

FILE 'EMBASE' ENTERED AT 13:00:42 ON 07 MAY 2002

FILE 'BIOSIS' ENTERED AT 13:00:42 ON 07 MAY 2002

FILE 'SCISEARCH' ENTERED AT 13:00:42 ON 07 MAY 2002

FILE 'CAPLUS' ENTERED AT 13:00:42 ON 07 MAY 2002


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L2        1034 L1 AND SH3 DOMAIN

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L3           2 L2 AND FORMIN BINDING PROTEIN

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L4 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
1999:412634 Document No. 131:54792 New human SH3-containing proteins and cDNAs and their therapeutic use. Bandman, Olga; Guegler, Karl J.; Lal, Preeti (Incyte Pharmaceuticals, Inc., USA). U.S. US 5916753 A 19990629, 32 pp. (English). CODEN: USXXAM. APPLICATION: US 1997-970133 19971113.

AB The invention is based on the discovery of two new human SH3-contg. proteins (HS3C), the polynucleotides encoding HS3C, and the use of these compns. for the diagnosis, prevention, or treatment of cancer and immune and developmental disorders. Nucleic acids encoding the HS3C-1 of the present invention were first identified in Incyte Clone 865744 from the brain tumor cDNA library (BRAITUT03) and the HS3C-2 in Incyte Clone 1816529 from the normal prostate tissue cDNA library (PROSNOT20) using a computer search for amino acid sequence alignments. Expression vectors, host cells, **antibodies**, agonists, and antagonists are also provided. Methods for treating or preventing disorders assocd. with expression of HS3C are described.

L4 ANSWER 2 OF 2 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:695715 The Genuine Article (R) Number: 116NB. WW domain-mediated interactions reveal a spliceosome-associated protein that binds a third class of proline-rich motif: The proline glycine and methionine-rich motif . Bedford M T; Reed R; Leder P (Reprint). HARVARD UNIV, SCH MED, DEPT GENET, BOSTON, MA 02115 (Reprint); HARVARD UNIV, SCH MED, DEPT GENET, BOSTON, MA 02115; HOWARD HUGHES MED INST, BOSTON, MA 02115; HARVARD UNIV, SCH MED, DEPT CELL BIOL, BOSTON, MA 02115. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (1 SEP 1998) Vol. 95, No. 18, pp. 10602-10607. Publisher: NATL ACAD SCIENCES. 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418. ISSN: 0027-8424. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pre-mRNA splicing requires the bridging of the 5' and 3' ends of the intron. In yeast, this bridging involves interactions between the WW domains in the splicing factor PRP40 and a proline-rich domain in the branchpoint binding protein, BBP. Using a proline-rich domain derived from formin (a product of the murine limb deformity locus), we have identified a family of murine **formin binding proteins** (FBP's), each of which contains one or more of a special class of tyrosine-rich WW domains. Two of these WW domains, in the proteins FBP11 and FBP21, are strikingly similar to those found in the yeast splicing factor PRP40. We show that FBP21 is present in highly purified spliceosomal complex A, is associated with U2 snRNPs, and colocalizes with splicing factors in nuclear speckle domains. Moreover, FBP21 interacts directly with the U1 snRNP protein U1C, the core snRNP proteins SmB and SmB', and the branchpoint binding protein SF1/mBBP. Thus, FBP21 may play a role in cross-intron bridging of U1 and U2 snRNPs in the mammalian A complex.

=> d his

(FILE 'HOME' ENTERED AT 13:00:29 ON 07 MAY 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 13:00:42 ON 07 MAY 2002

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L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
1999:412634 Document No. 131:54792 New human SH3-containing proteins and cDNAs and their therapeutic use. Bandman, Olga; Guegler, Karl J.; Lal, Preeti (Incyte Pharmaceuticals, Inc., USA). U.S. US 5916753 A 19990629, 32 pp. (English). CODEN: USXXAM. APPLICATION: US 1997-970133 19971113.

AB The invention is based on the discovery of two new human SH3-contg. proteins (HS3C), the polynucleotides encoding HS3C, and the use of these compns. for the diagnosis, prevention, or treatment of cancer and immune and developmental disorders. Nucleic acids encoding the HS3C-1 of the present invention were first identified in Incyte Clone 865744 from the brain tumor cDNA library (BRAITUT03) and the HS3C-2 in Incyte Clone 1816529 from the normal prostate tissue cDNA library (PROSNOT20) using a computer search for amino acid sequence alignments. Expression vectors, host cells, **antibodies**, agonists, and antagonists are also provided. Methods for treating or preventing disorders assocd. with expression of HS3C are described.

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L6 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
1999:412634 Document No. 131:54792 New human SH3-containing proteins and cDNAs and their therapeutic use. Bandman, Olga; Guegler, Karl J.; Lal, Preeti (Incyte Pharmaceuticals, Inc., USA). U.S. US 5916753 A 19990629, 32 pp. (English). CODEN: USXXAM. APPLICATION: US 1997-970133 19971113.

AB The invention is based on the discovery of two new human SH3-contg. proteins (HS3C), the polynucleotides encoding HS3C, and the use of these compns. for the diagnosis, prevention, or treatment of cancer and immune and developmental disorders. Nucleic acids encoding the HS3C-1 of the present invention were first identified in Incyte Clone 865744 from the brain tumor cDNA library (BRAITUT03) and the **HS3C-2** in Incyte Clone 1816529 from the normal prostate tissue cDNA library (PROSNOT20) using a computer search for amino acid sequence alignments. Expression vectors, host cells, **antibodies**, agonists, and antagonists are also provided. Methods for treating or preventing disorders assocd. with expression of HS3C are described.

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L9 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2002 ACS
2001:775265 Document No. 136:132090 Investigation of differentially expressed genes during the development of mouse cerebellum. Kagami, Yoshihiro; Furuichi, Teiichi (Laboratory for Molecular Neurogenesis, Brain Science Institute, RIKEN, Wako, 351-0198, Japan). Gene Expression Patterns, 1(1), 39-59 (English) 2001. CODEN: GEPEAD. ISSN: 1567-133X. Publisher: Elsevier Science B.V..
AB Before the discovery of DNA microarray and DNA chip technol., the expression of only a small no. of genes could be analyzed at a time. Currently, such technol. allows us the simultaneous anal. of a large no. of genes to systematically monitor their expression patterns that may be assocd. with various biol. phenomena. We utilized the Affymetrix GeneChip Mu11K to analyze the gene expression profile in developing mouse cerebellum to assist in the understanding of the genetic basis of cerebellar development in mice. Our anal. showed 81.6% (10.321/12.654) of the genes represented on the GeneChip were expressed in the postnatal cerebellum, and among those, 8.7% (897/10.321) were differentially expressed with more than a two-fold change in their max. and min. expression levels during the developmental time course. Further anal. of the differentially expressed genes that were clustered in terms of their expression patterns and the function of their encoded products revealed an aspect of the genetic foundation that lies beneath the cellular events and neural network formation that takes place during the development of the mouse cerebellum.

L9 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2002 ACS
2000:483900 Document No. 133:116451 Novel human protein containing drebrin-like sequence and **SH3 domain**, and its cDNA. Lill, Roland; Saeki, Mihoro; Kato, Seiji (Foundation for Scientific Technology Promotion, Japan). Jpn. Kokai Tokkyo Koho JP 2000197489 A2 20000718, 12 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1999-2254 19990107.
AB A novel human protein contg. drebrin-like sequence and **SH3 domain**, its cDNA, recombinant vector, and **antibody** to it, are disclosed. The putative amino acid sequence of the protein showed homol. to mouse **SH3 contg. protein** SH3P7 over the entire range, to human drebrin E2 over the N-terminal 300 amino acids, and to human c-src kinase **SH3 domain** over the

C-terminal 60 amino acids. Northern blot anal. showed a uniform tissue distribution. GFP fusion protein was expressed in E. coli.

L9 ANSWER 3 OF 14 MEDLINE DUPLICATE 1
2001063645 Document Number: 21013282. PubMed ID: 11127814. Meltrin alpha cytoplasmic domain interacts with **SH3 domains** of Src and Grb2 and is phosphorylated by v-Src. Suzuki A; Kadota N; Hara T; Nakagami Y; Izumi T; Takenawa T; Sabe H; Endo T. (Department of Biology, Faculty of Science, Chiba University, Japan.) ONCOGENE, (2000 Nov 30) 19 (51) 5842-50. Journal code: ONC. ISSN: 0950-9232. Pub. country: England: United Kingdom. Language: English.

AB Meltrin alpha/ADAM12 is a member of the ADAM/MDC family proteins characterized by the presence of metalloprotease and disintegrin domains. This protein also contains a single transmembrane domain and a relatively long cytoplasmic domain containing several proline-rich sequences. These sequences are compatible with the consensus sequences for binding the Src homology 3 (**SH3**) **domains**. To determine whether the proline-rich sequences interact with **SH3 domains** in several proteins, binding of recombinant **SH3 domains** to the meltrin alpha cytoplasmic domain was analysed by pull-down assays. The **SH3 domains** of Src and Yes bound strongly, but that of Abl or phosphatidylinositol 3-kinase p85 subunit did not. Full-length Grb2/Ash bound strongly, whereas its N-terminal **SH3 domain** alone did less strongly. Src and Grb2 in bovine brain extracts also bound to meltrin alpha cytoplasmic domain on affinity resin. Furthermore, immunoprecipitation with a monoclonal **antibody** to meltrin alpha resulted in coprecipitation of Src and Grb2 with meltrin alpha in cell extracts, suggesting that Src and Grb2 are associated in vivo with meltrin alpha cytoplasmic domain. This notion was also supported by the findings that exogenously expressed meltrin cytoplasmic domain coexisted with Src and Grb2 on the membrane ruffles. The C-terminal Tyr901 of meltrin alpha was phosphorylated both in vitro and in cultured cells by v-Src. These results may imply that meltrin alpha cytoplasmic domain is involved in a signal transduction for some biological function through the interaction with **SH3-containing proteins**.

L9 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2002 ACS
1999:412634 Document No. 131:54792 New human **SH3-containing proteins** and cDNAs and their therapeutic use. Bandman, Olga; Guegler, Karl J.; Lal, Preeti (Incyte Pharmaceuticals, Inc., USA). U.S. US 5916753 A 19990629, 32 pp. (English). CODEN: USXXAM. APPLICATION: US 1997-970133 19971113.

AB The invention is based on the discovery of two new human **SH3-contg. proteins** (HS3C), the polynucleotides encoding HS3C, and the use of these compns. for the diagnosis, prevention, or treatment of cancer and immune and developmental disorders. Nucleic acids encoding the HS3C-1 of the present invention were first identified in Incyte Clone 865744 from the brain tumor cDNA library (BRAITUT03) and the HS3C-2 in Incyte Clone 1816529 from the normal prostate tissue cDNA library (PROSNOT20) using a computer search for amino acid sequence alignments. Expression vectors, host cells, **antibodies**, agonists, and antagonists are also provided. Methods for treating or preventing disorders assocd. with expression of HS3C are described.

L9 ANSWER 5 OF 14 MEDLINE DUPLICATE 2
1999211957 Document Number: 99211957. PubMed ID: 10194451. A novel SH2-containing phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase (SHIP2) is constitutively tyrosine phosphorylated and associated with src homologous and collagen gene (SHC) in chronic myelogenous leukemia progenitor cells. Wisniewski D; Strife A; Swendeman S; Erdjument-Bromage H; Geromanos S; Kavanaugh W M; Tempst P; Clarkson B. (Sloan-Kettering Institute for Cancer Research, Molecular Pharmacology and Therapeutics Program and Molecular Biology Program, New York, NY, USA.) BLOOD, (1999

Apr 15) 93 (8) 2707-20. Journal code: A8G; 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Because of the probable causal relationship between constitutive p210(bcr/abl) protein tyrosine kinase activity and manifestations of chronic-phase chronic myelogenous leukemia (CML; myeloid expansion), a key goal is to identify relevant p210 substrates in primary chronic-phase CML hematopoietic progenitor cells. We describe here the purification and mass spectrometric identification of a 155-kD tyrosine phosphorylated protein associated with src homologous and collagen gene (SHC) from p210(bcr/abl)-expressing hematopoietic cells as SHIP2, a recently reported, unique SH2-domain-containing protein closely related to phosphatidylinositol polyphosphate 5-phosphatase SHIP. In addition to an N-terminal SH2 domain and a central catalytic region, SHIP2 (like SHIP1) possesses both potential PTB(NPXY) and **SH3 domain** (PXXP) binding motifs. Thus, two unique 5-ptases with striking structural homology are coexpressed in hematopoietic progenitor cells. Stimulation of human hematopoietic growth factor responsive cell lines with stem cell factor (SCF), interleukin-3 (IL-3), and granulocyte-macrophage colony-stimulating factor (GM-CSF) demonstrate the rapid tyrosine phosphorylation of SHIP2 and its resulting association with SHC. This finding suggests that SHIP2, like that reported for SHIP1 previously, is linked to downstream signaling events after activation of hematopoietic growth factor receptors. However, using **antibodies** specific to these two proteins, we demonstrate that, whereas SHIP1 and SHIP2 selectively hydrolyze PtdIns(3,4,5)P3 in vitro, only SHIP1 hydrolyzes soluble Ins(1,3,4,5)P4. Such an enzymatic difference raises the possibility that SHIP1 and SHIP2 may serve different functions. Preliminary binding studies using lysates from p210(bcr/abl)-expressing cells indicate that both Ptyr SHIP2 and Ptyr SHIP1 bind to the PTB domain of SHC but not to its SH2 domain. Interestingly, SHIP2 was found to selectively bind to the **SH3 domain** of ABL, whereas SHIP1 selectively binds to the **SH3 domain** of Src. Furthermore, in contrast to SHIP1, SHIP2 did not bind to either the N-terminal or C-terminal **SH3 domains** of GRB2. These observations suggest (1) that SHIP1 and SHIP2 may have a different hierarchy of binding **SH3 containing proteins** and therefore may modulate different signaling pathways and/or localize to different cellular compartments and (2) that they may be substrates for tyrosine phosphorylation by different tyrosine kinases. Because recent evidence has clearly implicated both PI(3,4, 5)P3 and PI(3,4)P2 in growth factor-mediated signaling, our finding that both SHIP1 and SHIP2 are constitutively tyrosine phosphorylated in CML primary hematopoietic progenitor cells may thus have important implications in p210(bcr/abl)-mediated myeloid expansion.

L9 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2002 ACS

1998:714431 Document No. 130:91803 Characterization of a novel **SH3 -containing protein** that may interact with connexin43.

Jin, Chengshi; Lau, Alan F. (Molecular Carcinogenesis, Cancer Research Center, and Department of Genetics and Molecular Biology, School of Medicine, University of Hawaii at Manoa, Honolulu, HI, 96813, USA). Gap Junctions, Proceedings of the International Gap Junction Conference, 8th, Key Largo, Fla., July 12-17, 1997, Meeting Date 1997, 230-234. Editor(s): Werner, Rudolf. IOS Press: Amsterdam, Neth. (English) 1998. CODEN: 66XYAX.

AB We searched for putative Cx43 interacting proteins using a two-hybrid screen of an embryonic mouse cDNA library. A cDNA fragment (CID62) encoding a **SH3 domain** was identified in this screening. We isolated a 2.7-Kb long full length cDNA (CIP62) using CID62 as a probe. The CIP62 cDNA was predicted to encode a 760 aa protein without significant homol. to proteins of known function. To further characterize the protein, flag-tagged CIP62 was expressed in Spodoptera frugiperda (Sf9) cells. We detected an approx. 83-kDa protein by Western

blot anal. using anti-Flag **antibody**. We obsd. that this protein was predominantly localized to the plasma membrane of Sf9 cells at the immunofluorescence microscopic level.

L9 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2002 ACS

1997:633486 Document No. 127:306217 Wiskott-Aldrich syndrome/X-linked thrombocytopenia: WASP gene mutations, protein expression, and phenotype. Zhu, Qili; Watanabe, Chiaki; Liu, Ting; Hollenbaugh, Diane; Blaese, R. Michael; Kanner, Steven B.; Aruffo, Alejandro; Ochs, Hans D. (Div. Infectious Diseases, Immunol. Rheumatol., Dep. Pediatrics, Univ. Washington Sch. Med., Seattle, WA, USA). Blood, 90(7), 2680-2689 (English) 1997. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: Saunders.

AB Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT), caused by mutations of the WAS protein (WASP) gene, represent different phenotypes of the same disease. To demonstrate a phenotype/genotype correlation, was detd. WASP gene mutations in 48 unrelated WAS families. Mutations included missense (20 families) and nonsense (eight) mutations located mostly in exons 1 to 4, and splice-site mutations (seven) and deletions and insertions (13) located preferentially in exons 7 to 11. Both genomic DNA and cDNA were sequenced and WASP expression was measured in cell lysates using peptide-specific anti-WASP **antibodies**. WASP was expressed in hematopoietic cell lines including bone marrow-derived CD34+ cells. Missense mutation located in exons 1 to 3 caused mild disease in all but one family and permitted WASP expression, although frequently at decreased concn. Missense mutations affecting exon 4 were assocd. with classic WAS and, with one exception, barely detectable WASP. Nonsense mutations caused classic WAS and lack of protein. Insertions, deletions, and splice-site mutations resulted in classic WAS and absent, unstable, truncated, or multiply spliced protein. Using affinity pptn., WASP was found to bind to Src **SH3-contg. proteins** Fyn, Lck, PLC-.gamma., and Grb2, and mutated WASP, if expressed, was able to bind to Fyn-glutathione S-transferase (GST) fusion protein. We conclude that missense mutations affecting the PH domain (exons 1 to 3) of WASP inhibit less important functions of the protein and result in a mild phenotype, and that missense mutations affecting exon 4 and complex mutations affecting the 3' portion of WASP interfere with crucial functions of the protein and cause classic WAS.

L9 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2002 ACS

1994:452604 Document No. 121:52604 C3G, a guanine nucleotide-releasing protein expressed ubiquitously, binds to the Src homology 3 domains of CRK and GRB2/ASH proteins. Tanaka, Shinya; Morishita, Takashi; Hashimoto, Yuko; Hattori, Seisuke; Nakamura, Shun; Shibuya, Masabumi; Matuoka, Koozi; Takenawa, Tadaomi; Kurata, Takeshi; et al. (Dep. Pathology, Hokkaido Univ., Sapporo, 060, Japan). Proc. Natl. Acad. Sci. U. S. A., 91(8), 3443-7 (English) 1994. CODEN: PNASA6. ISSN: 0027-8424.

AB CRK protein, together with GRB2/ASH and Nck proteins, belongs to the adaptor-type Src homol. (SH)2-contg. mols., which transduce signals from tyrosine kinases. Here, another guanine nucleotide-releasing protein (GNRP), C3G, was identified as a CRK SH3-binding protein. The nucleotide sequence of a 4.10-kb C3G cDNA contains a 3.2-kb open reading frame encoding a 121-kDa protein, and **antibodies** against C3G detect a protein of 130-140 kDa. The C-terminus of C3G has a peptide sequence homologous to GNRPs for Ras, and the expression of this C-terminus region suppresses the loss of CDC25 function in the yeast Saccharomyces cerevisiae. The C3G protein expressed in Escherichia coli binds to CRK and GRB2/ASH proteins. Mutational anal. of C3G assigns the SH3 binding region to a 50-amino acid region contg. a proline-rich sequence. The mRNAs of both the C3G and CRK proteins are expressed ubiquitously in human adult and fetal tissues. The results of these studies suggest that the complex of CRK and C3G, or GRB2/ASH and C3G, may transduce the signals from tyrosine kinases to Ras in a no. of different tissues.

L9 ANSWER 9 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
94:693715 The Genuine Article (R) Number: PP021. IDENTIFICATION OF CRKL AS THE CONSTITUTIVELY PHOSPHORYLATED 39-KD TYROSINE PHOSPHOPROTEIN IN CHRONIC MYELOGENOUS LEUKEMIA-CELLS. NICHOLS G L (Reprint); RAINES M A; VERA J C; LACOMIS L; TEMPST P; GOLDE D W. MEM SLOAN KETTERING CANC CTR, DIV HEMATOL ONCOL, PROGRAM MOLEC PHARMACOL & THERAPEUT, BOX 492, NEW YORK, NY, 10021 (Reprint); MEM SLOAN KETTERING CANC CTR, PROGRAM MOLEC BIOL, NEW YORK, NY, 10021. BLOOD (01 NOV 1994) Vol. 84, No. 9, pp. 2912-2918. ISSN: 0006-4971. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chronic myelogenous leukemia (CML) is characterized by the presence of the Philadelphia (Ph) chromosome in clonally derived hematopoietic precursors and their progeny. The Ph chromosome arises from a translocation that deregulates the c-ABL protein tyrosine kinase, giving it transforming potential and increased kinase activity. We observed a unique 39-kD tyrosine phosphoprotein (pp39), previously reported in blastic CML cell lines, in neutrophils from 50 cases of chronic phase CML. This protein was prominently and constitutively tyrosine-phosphorylated in CML neutrophils and was not phosphorylated in normal neutrophils. Stimulation of normal neutrophils with cytokines and agonists did not induce tyrosine phosphorylation of proteins migrating in the region of pp39, and the phosphorylation state of pp39 in CML neutrophils was not affected by kinase inhibitors known to downregulate the ABL kinase. The pp39 was not phosphorylated in hematopoietic cells from healthy donors or from patients with Ph chromosome-negative myeloproliferative disorders. Using micro amino acid sequencing of purifled preparations of pp39, we identified pp39 as CRKL protein, which is consistent with recent immunologic studies in the blastic K562 cell line. Immunoblotting with anti-CRKL **antibodies** showed the presence of CRKL protein in CML cells and cell lines as well as in antiphosphotyrosine immunoprecipitates from CML cells. Our results suggest that pp39 CRKL in CML neutrophils may be stably tyrosine-phosphorylated by the BCR/ABL kinase at an early stage of myeloid differentiation when the ABL kinase is active. CRK, CRKL, and other SH2 (SRC homology domain)/**SH3-containing proteins** function as adaptor molecules in nonreceptor tyrosine kinase signalling pathways. Although the CRKL protein is present in normal neutrophils, it is not tyrosine-phosphorylated, and the inability to induce such phosphorylation in normal neutrophils suggests a special role of this phosphoprotein in the pathogenesis of CML. Constitutive phosphorylation of CRKL is unique to CML, indicating that it may be a useful target for therapeutic intervention. (C) 1994 by The American Society of Hematology.

L9 ANSWER 10 OF 14 MEDLINE
95053744 Document Number: 95053744. PubMed ID: 7525847. IL-5 receptor-mediated tyrosine phosphorylation of SH2/**SH3-containing proteins** and activation of Bruton's tyrosine and Janus 2 kinases. Sato S; Katagiri T; Takaki S; Kikuchi Y; Hitoshi Y; Yonehara S; Tsukada S; Kitamura D; Watanabe T; Witte O; +. (Department of Immunology, University of Tokyo, Japan.) JOURNAL OF EXPERIMENTAL MEDICINE, (1994 Dec 1) 180 (6) 2101-11. Journal code: I2V; 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Interleukin 5 (IL-5) induces proliferation and differentiation of B cells and eosinophils by interacting with its receptor (IL-5R) which consists of two distinct polypeptide chains, alpha and beta (beta c). Although both IL-5R alpha and beta c lack a kinase catalytic domain, IL-5 is capable of inducing tyrosine phosphorylation of cellular proteins. We investigated the role of IL-5R alpha in tyrosine phosphorylation of molecules involved in IL-5 signal transduction, using an IL-5-dependent early B cell line, Y16 and transfectants expressing intact or mutant IL-5R alpha together with intact beta c. The results revealed that the transfectants expressing truncated IL-5R alpha, which entirely lacks a cytoplasmic domain, together with beta c, showed neither protein-tyrosine phosphorylation nor

proliferation in response to IL-5. This confirms that IL-5R alpha plays a critical role in protein-tyrosine phosphorylation which triggers cell growth. IL-5 stimulation results in rapid tyrosine phosphorylation of beta c and proteins containing Src homology 2 (SH2) and/or **SH3 domains** such as phosphatidyl-inositol-3 kinase, Shc, Vav, and HS1, suggesting their involvement in IL-5-mediated signal transduction. IL-5 stimulation significantly enhanced activities of Janus 2 and B cell-specific Bruton's tyrosine kinases (JAK2 and Btk) and increased the tyrosine phosphorylation of JAK2 kinase. These results and recent data on signaling of growth factors taken together, multiple biochemical pathways driven by tyrosine kinases such as JAK2 and Btk are involved in IL-5 signal transduction.

L9 ANSWER 11 OF 14 MEDLINE

94067150 Document Number: 94067150. PubMed ID: 8247004. Identification and sequence analysis of cDNAs encoding a 110-kilodalton actin filament-associated pp60src substrate. Flynn D C; Leu T H; Reynolds A B; Parsons J T. (Department of Microbiology, School of Medicine, University of Virginia, Charlottesville 22908.) MOLECULAR AND CELLULAR BIOLOGY, (1993 Dec) 13 (12) 7892-900. Journal code: NGY; 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB Transformation of chicken embryo cells by oncogenic forms of pp60src (e.g., pp60v-src or pp60527F) is linked with a concomitant increase in the steady-state levels of tyrosine-phosphorylated cellular proteins. Activated forms of the Src protein-tyrosine kinase stably associate with tyrosine-phosphorylated proteins, including a protein of 110 kDa, pp110. Previous reports have established that stable complex formation between pp110 and pp60src requires the structural integrity of the Src SH2 and **SH3 domains**, whereas tyrosine phosphorylation of pp110 requires only the structural integrity of the **SH3 domain**. In normal chicken embryo cells, pp110 colocalizes with actin stress filaments, and in Src-transformed cells, pp110 is found associated with podosomes (rosettes). Here, we report the identification and characterization of cDNAs encoding pp110. The predicted open reading frame encodes a polypeptide of 635 amino acids which exhibits little sequence similarity with other protein sequences present in the available sequence data bases. Thus, pp110 is a distinctive cytoskeleton-associated protein. On the basis of its association with actin stress filaments, we propose the term AFAP-110, for actin filament-associated protein of 110 kDa. In vitro analysis of AFAP-110 binding to bacterium-encoded glutathione S-transferase (GST) fusion proteins revealed that AFAP-110 present in normal cell extracts binds efficiently to Src SH3/SH2-containing fusion proteins, less efficiently to Src **SH3-containing proteins**, and poorly to SH2-containing fusion proteins. In contrast, AFAP-110 in Src-transformed cell extracts bound to GST-SH3/SH2 and GST-SH2 fusion proteins. Analysis of AFAP-110 cDNA sequences revealed the presence of sequence motifs predicted to bind to SH2 and **SH3 domains**, respectively.(ABSTRACT TRUNCATED AT 250 WORDS)

L9 ANSWER 12 OF 14 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1993:500727 Document No.: PREV199396124734. Ash/Grb-2, a SH2/**SH3-containing protein**, couples to signaling for mitogenesis and cytoskeletal reorganization by EGF and PDGF. Matuoka, K.; Shibasaki, F.; Shibata, M.; Takenawa, T.. Dep. Biosignal Res., Tokyo Metropolitan Inst. Gerontol., Itabashi-ku, Tokyo 173 Japan. EMBO (European Molecular Biology Organization) Journal, (1993) Vol. 12, No. 9, pp. 3473-3473. ISSN: 0261-4189. Language: English.

AB The Src homology (SH) region 2 binds to phosphorylated tyrosine residues and **SH3 domains** may interact with cytoskeletal molecules and GTPase-activating proteins for Rho/Rac proteins (the small GTP-binding proteins related to Ras). The recently cloned Ash/Grb-2 protein, a 25-28 kDa molecule composed entirely of SH2 and **SH3 domains**, is a mammalian homolog of the Caenorhabditis elegans

Sem-5 protein, which communicates between a receptor protein tyrosine kinase and a Ras protein. In the present study the function of Ash/Grb-2 was investigated by microinjecting cells with an anti-Ash **antibody** . The **antibody** abolished both S phase entry and the reorganization of actin assembly to ruffle formation upon stimulation with epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). On the other hand, anti-Ash **antibody** had no effect on S phase entry or actin stress fiber formation induced by either serum or lysophosphatidic acid. Since the induction of DNA synthesis, ruffle induction and stress fiber formation involve a function of Ras, Rac activation and Rho activation respectively, the findings strongly suggest that Ash plays a critical role in the signaling of both pathways downstream from growth factor receptors to Ras and Rac. Consistent with this, Ash co-precipitated with EGF receptor from EGF-stimulated cells. Other proteins of approximately 21, 29, 135 and 160 kDa were also detected in the anti-Ash **antibody** immunoprecipitates, suggesting a role of Ash as a linker molecule in signal transduction downstream of growth factor receptors.

L9 ANSWER 13 OF 14 MEDLINE DUPLICATE 3

94074546 Document Number: 94074546. PubMed ID: 8253073. Ash/Grb-2, a SH2/**SH3-containing protein**, couples to signaling for mitogenesis and cytoskeletal reorganization by EGF and PDGF. Matuoka K; Shibasaki F; Shibata M; Takenawa T. (Department of Biosignal Research, Tokyo Metropolitan Institute of Gerontology, Japan.) EMBO JOURNAL, (1993 Sep) 12 (9) 3467-73. Journal code: EMB; 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The Src homology (SH) region 2 binds to phosphorylated tyrosine residues and **SH3 domains** may interact with cytoskeletal molecules and GTPase-activating proteins for Rho/Rac proteins (the small GTP-binding proteins related to Ras). The recently cloned Ash/Grb-2 protein, a 25-28 kDa molecule composed entirely of SH2 and **SH3 domains**, is a mammalian homolog of the Caenorhabditis elegans Sem-5 protein, which communicates between a receptor protein tyrosine kinase and a Ras protein. In the present study the function of Ash/Grb-2 was investigated by microinjecting cells with an anti-Ash **antibody** . The **antibody** abolished both S phase entry and the reorganization of actin assembly to ruffle formation upon stimulation with epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). On the other hand, anti-Ash **antibody** had no effect on S phase entry or actin stress fiber formation induced by either serum or lysophosphatidic acid. Since the induction of DNA synthesis, ruffle induction and stress fiber formation involve a function of Ras, Rac activation and Rho activation respectively, the findings strongly suggest that Ash plays a critical role in the signaling of both pathways downstream from growth factor receptors to Ras and Rac. Consistent with this, Ash co-precipitated with EGF receptor from EGF-stimulated cells. Other proteins of approximately 21, 29, 135 and 160 kDa were also detected in the anti-Ash **antibody** immunoprecipitates, suggesting a role of Ash as a linker molecule in signal transduction downstream of growth factor receptors.

L9 ANSWER 14 OF 14 MEDLINE DUPLICATE 4

93078786 Document Number: 93078786. PubMed ID: 1448108. The SH2/**SH3 domain**-containing protein Nck is recognized by certain anti-phospholipase C-gamma 1 monoclonal **antibodies**, and its phosphorylation on tyrosine is stimulated by platelet-derived growth factor and epidermal growth factor treatment. Meisenhelder J; Hunter T. (Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California 92186.) MOLECULAR AND CELLULAR BIOLOGY, (1992 Dec) 12 (12) 5843-56. Journal code: NGY; 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB In the course of our investigation of phospholipase C (PLC)-gamma 1

phosphorylation by using a set of anti-PLC-gamma 1 monoclonal **antibodies** (P.-G. Suh, S. H. Ryu, W. C. Choi, K.-Y. Lee, and S. G. Rhee, J. Biol. Chem. 263:14497-14504, 1988), we found that some of these **antibodies** directly recognize a 47-kDa protein. We show here that this 47-kDa protein is identical to the SH2/**SH3**-**containing protein** Nck (J. M. Lehmann, G. Riethmuller, and J. P. Johnson, Nucleic Acids Res. 18:1048, 1990). Nck was found to be constitutively phosphorylated on serine in resting NIH 3T3 cells. Platelet-derived growth factor (PDGF) treatment led to increased Nck phosphorylation on both tyrosine and serine. Nck was also found to be phosphorylated on tyrosine in epidermal growth factor (EGF)-treated A431 cells and in v-Src-transformed NIH 3T3 cells. Multiple sites of serine phosphorylation were detected in Nck from resting cells, and no novel sites were found upon PDGF or EGF treatment. A single major tyrosine phosphorylation site was found in Nck in both PDGF- and EGF-treated cells and in v-Src-transformed cells. This same tyrosine was phosphorylated in vitro by purified PDGF and EGF receptors and also by pp60c-src. We compared the phosphorylation of Nck and PLC-gamma 1 in several cell lines transformed by oncogenes with different modes of transformation. Although PLC-gamma 1 and Nck have significant amino acid identity, particularly in their SH3 regions, and both associate with growth factor receptors in a ligand-dependent manner, they were not always phosphorylated on tyrosine in a coincident manner.

```
=> s l2 and monoclona;
L10           0 L2 AND MONOCLONA

=> s l2 and monoclona;
L11           0 L2 AND MONOCLONA

=> s l2 and monoclonal
L12         279 L2 AND MONOCLONAL

=> s l12 and "proline rich phosphoprotein"
L13           0 L12 AND "PROLINE RICH PHOSPHOPROTEIN"

=> s l2 and "SH3 domain"
L14        1034 L2 AND "SH3 DOMAIN"

=> dup remove l13
L13 HAS NO ANSWERS

=> dup remove l14
PROCESSING COMPLETED FOR L14
L15         443 DUP REMOVE L14 (591 DUPLICATES REMOVED)

=> s l15 and FBP1
L16           0 L15 AND FBP1

=> s l15 and "FBP1"
L17           0 L15 AND "FBP1"

=> s l15 and "FBP17"
L18           1 L15 AND "FBP17"

=> d l18 cbib abs

L18  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
1999:412634   Document No. 131:54792  New human SH3-containing proteins and
     cDNAs and their therapeutic use.  Bandman, Olga; Guegler, Karl J.; Lal,
     Preeti (Incyte Pharmaceuticals, Inc., USA).  U.S. US 5916753 A  19990629,
     32 pp.  (English).  CODEN: USXXAM.  APPLICATION: US 1997-970133 19971113.
```

AB The invention is based on the discovery of two new human SH3-contg. proteins (HS3C), the polynucleotides encoding HS3C, and the use of these compns. for the diagnosis, prevention, or treatment of cancer and immune and developmental disorders. Nucleic acids encoding the HS3C-1 of the present invention were first identified in Incyte Clone 865744 from the brain tumor cDNA library (BRAITUT03) and the HS3C-2 in Incyte Clone 1816529 from the normal prostate tissue cDNA library (PROSNOT20) using a computer search for amino acid sequence alignments. Expression vectors, host cells, **antibodies,** agonists, and antagonists are also provided. Methods for treating or preventing disorders assocd. with expression of HS3C are described.

```
=> s l15 and "FBP27"
L19           0 L15 AND "FBP27"

=> d his

     (FILE 'HOME' ENTERED AT 13:00:29 ON 07 MAY 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 13:00:42 ON
     07 MAY 2002
L1       2302080 S ANTIBOD?
L2          1034 S L1 AND SH3 DOMAIN
L3             2 S L2 AND FORMIN BINDING PROTEIN
L4             2 DUP REMOVE L3 (0 DUPLICATES REMOVED)
L5             1 S L2 AND "FBP17"
L6             1 S L2 AND "HS3C-2"
L7             0 S L2 AND "ALYTF"
L8            28 S L2 AND "SH3 CONTAINING PROTEIN"
L9            14 DUP REMOVE L8 (14 DUPLICATES REMOVED)
L10            0 S L2 AND MONOCLONA
L11            0 S L2 AND MONOCLONA
L12          279 S L2 AND MONOCLONAL
L13            0 S L12 AND "PROLINE RICH PHOSPHOPROTEIN"
L14         1034 S L2 AND "SH3 DOMAIN"
L15          443 DUP REMOVE L14 (591 DUPLICATES REMOVED)
L16            0 S L15 AND FBP1
L17            0 S L15 AND "FBP1"
L18            1 S L15 AND "FBP17"
L19            0 S L15 AND "FBP27"

=> s l2 and "WWPWW motif"
L20           0 L2 AND "WWPWW MOTIF"

=> s bandman o?/au or guegler k?/au or Lal p?/au
L21       1383 BANDMAN O?/AU OR GUEGLER K?/AU OR LAL P?/AU

=> s l21 and antibody
L22         626 L21 AND ANTIBODY

=> s l22 and formins
L23           0 L22 AND FORMINS

=> dup remove l22
PROCESSING COMPLETED FOR L22
L24          569 DUP REMOVE L22 (57 DUPLICATES REMOVED)

=> s l24 and "SH3"
L25           2 L24 AND "SH3"

=> d l25 1-2 cbib abs
```

L25 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:74092 Document No.: PREV200200074092. **SH3**-containing proteins.
Bandman, Olga; Guegler, Karl J.; Lal, Preeti.
ASSIGNEE: Incyte Genomics Inc., Palo Alto, CA, USA. Patent Info.: US 6326158 December 04, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 4, 2001) Vol. 1253, No. 1, pp. No Pagination. ftp://ftp.uspto.gov/pub/patdata/. e-file. ISSN: 0098-1133. Language: English.

AB The invention provides a human **SH3**-containing protein (HS3C) and polynucleotides which identify and encode HS3C. The invention also provides expression vectors, host cells, **antibodies**, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of HS3C.

L25 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
1999:412634 Document No. 131:54792 New human **SH3**-containing proteins and cDNAs and their therapeutic use. **Bandman, Olga; Guegler, Karl J.; Lal, Preeti** (Incyte Pharmaceuticals, Inc., USA). U.S. US 5916753 A 19990629, 32 pp. (English). CODEN: USXXAM. APPLICATION: US 1997-970133 19971113.

AB The invention is based on the discovery of two new human **SH3** -contg. proteins (HS3C), the polynucleotides encoding HS3C, and the use of these compns. for the diagnosis, prevention, or treatment of cancer and immune and developmental disorders. Nucleic acids encoding the HS3C-1 of the present invention were first identified in Incyte Clone 865744 from the brain tumor cDNA library (BRAITUT03) and the HS3C-2 in Incyte Clone 1816529 from the normal prostate tissue cDNA library (PROSNOT20) using a computer search for amino acid sequence alignments. Expression vectors, host cells, **antibodies**, agonists, and antagonists are also provided. Methods for treating or preventing disorders assocd. with expression of HS3C are described.

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NEWS 4 Feb 01 DKILIT now produced by FIZ Karlsruhe and has a new update

frequency
NEWS 5 Feb 19 Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6 Mar 08 Gene Names now available in BIOSIS
NEWS 7 Mar 22 TOXLIT no longer available
NEWS 8 Mar 22 TRCTHERMO no longer available
NEWS 9 Mar 28 US Provisional Priorities searched with P in CA/CAplus and USPATFULL
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NEWS 13 Apr 09 BEILSTEIN: Reload and Implementation of a New Subject Area
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NEWS 15 Apr 19 US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 16 Apr 22 Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 17 Apr 22 BIOSIS Gene Names now available in TOXCENTER
NEWS 18 Apr 22 Federal Research in Progress (FEDRIP) now available

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	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 10:22:50 ON 08 MAY 2002

FILE 'EMBASE' ENTERED AT 10:22:50 ON 08 MAY 2002

FILE 'BIOSIS' ENTERED AT 10:22:50 ON 08 MAY 2002

FILE 'SCISEARCH' ENTERED AT 10:22:50 ON 08 MAY 2002

FILE 'CAPLUS' ENTERED AT 10:22:50 ON 08 MAY 2002

```
=> s antibod?
L1     2302220 ANTIBOD?

=> s l1 and "TRIPS"
L2          42 L1 AND "TRIPS"

=> s l2 and thyroid receptor interacting proteins
L3           0 L2 AND THYROID RECEPTOR INTERACTING PROTEINS

=> d l2

L2   ANSWER 1 OF 42     MEDLINE
AN   2002113623     MEDLINE
DN   21835028   PubMed ID: 11845521
TI   Asymptomatic amoebic infection: Entamoeba histolytica or Entamoeba dispar?
     That is the question.
AU   Gatti S; Petithory J C; Ardoin F; Pannetier C; Scaglia M
CS   Parasitology Laboratory, Virology Service, IRCCS San Matteo, Pavia, Italy.
SO   BULLETIN DE LA SOCIETE DE PATHOLOGIE EXOTIQUE, (2001 Nov) 94 (4) 304-7.
     Journal code: 9212564.
CY   France
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200203
ED   Entered STN: 20020216
     Last Updated on STN: 20020319
     Entered Medline: 20020318


=> s l1 and Cdc42
L4         417 L1 AND CDC42

=> s l4 and monoclonal
L5          61 L4 AND MONOCLONAL

=> s l5 and polyclonal
L6           1 L5 AND POLYCLONAL

=> d l6 cbib abs

L6   ANSWER 1 OF 1  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:153051  Document No.: PREV200200153051. Cdc42, Rac1, and Rac2
     expression and GTP-binding in normal and chronic myelogenous leukemia
     (CML) CD34+ hematopoietic progenitor cells (HPC. Brunstein, Claudio G.
     (1); Verfaillie, Catherine M. (1). (1) Medicine, University of Minnesota,
     Minneapolis, MN USA. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1,
     pp. 142a. http://www.bloodjournal.org/. print. Meeting Info.: 43rd Annual
     Meeting of the American Society of Hematology, Part 1 Orlando, Florida,
     USA December 07-11, 2001 ISSN: 0006-4971. Language: English.
AB   We and others have shown that the BCR/ABL fusion protein product, a
     constitutionally active tyrosine kinase, interferes with normal
     beta-1-integrin signaling pathways leading to decreased adhesion and
     increased migration of CML cells. The RhoGTPases Cdc42 and Rac
     regulate cytoskeleton remodeling, adhesion, and migration of murine
     hematopoietic stem cells, and also have been implicated in the control of
     proliferation and gene expression. Elimination of Rac2, the predominant
     form of Rac in hematopoietic cells, causes decreased migration of HPC in
     mouse and decreased phagocyte function and activation in humans. We
     studied the expression and GTP-loading of Cdc42, Rac1, and Rac2
     in Bcr/Abl positive and negative hematopoietic cells lines, and in human
     normal and CML derived CD34-positive cells. For the western-blots we used
     mouse monoclonal anti-Cdc42 and anti-Rac1 (BD
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Transduction Laboratories, Lexington, KY) and rabbit **polyclonal** anti-Rac2 (with a kind gift from Dr. G. Bokoch, The Scripps Research Institute). The pull-down assay was performed with the commercially available GST fusion-protein, corresponding to the p21-binding domain of human PAK-1 (**Cdc42**/Rac Activation Assays Kit, Upstate Biotechnology, Lake Placid, NY). In human mononuclear and CD34+ HPC from normal donors and CML patients in chronic phase the levels of **Cdc42**, Rac1, and Rac2 protein were similar. CML peripheral blood mononuclear cells contained increased active GTP bound **Cdc42** and decreased amounts of active Rac1 and Rac2 when compared to normal cells. Whereas increased active, GTP-loaded **Cdc42** was present in CML CD34+ cells compared to normal CD34+ HPC, the decrease in active, GTP-bound Rac1 and Rac2 was less prominent. Upon engagement of beta-1-integrins with a blocking **monoclonal antibody**, GTP loading of **Cdc42**, Rac1 and Rac2 increased in CD34+ HPC. Unexpectedly, the activation of **Cdc42**, Rac1 and Rac2 decreased in CML HPC following integrin engagement. Treatment of CML CD34+ HPC with 1 micromolar of the tyrosine kinase inhibitor STI571 (Novartis, Basel, Switzerland) overnight, restored "normal" activation of GTPases in the absence of integrin activation (i.e. lowered GTP-bound protein), and upregulation of GTP loading following beta-1-integrin engagement in CML CD34+ HPC. These studies show that (1) presence of Bcr/Abl leads to spontaneous activation of the GTPase **Cdc42**, but downregulates Rac1, and Rac2 GTP loading, which may contribute to the abnormal spontaneous migration and the abnormal baseline skeletal activation in CML CD34+ HPC. (2) In CML CD34+ HPC, GTPase activation seems to be decreased following integrin engagement, which may contribute to the decreased adhesion to and migration over fibronectin. (3) As these phenomena are blocked by STI571, the effect of Bcr/Abl is mediated by its tyrosine kinase fucntion. Whether inhibition of GTPase activitiy will restore normal hematopoietic cell function in CML is currently being studied.

```
=> dup remove l5
PROCESSING COMPLETED FOR L5
L7            41 DUP REMOVE L5 (20 DUPLICATES REMOVED)

=> s l7 and SH3
L8             2 L7 AND SH3

=> dup remove l8
PROCESSING COMPLETED FOR L8
L9             2 DUP REMOVE L8 (0 DUPLICATES REMOVED)

=> d l9 1-2 cbib abs

L9   ANSWER 1 OF 2     MEDLINE
1999422020  Document Number: 99422020.    PubMed ID: 10490822.   Ras-GTPase
     activating protein inhibition specifically induces apoptosis of tumour
     cells. Leblanc V; Delumeau I; Tocque B. (ExonHit Therapeutics, 65 Bld
     Massena, 75013 Paris, France. ) ONCOGENE, (1999 Aug 26) 18 (34) 4884-9.
     Journal code: ONC; 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United
     Kingdom. Language: English.
AB   Oncogenes and tumour suppressor genes control the balance between
     apoptotic death and anti-apoptotic survival signals determining whether a
     cell proliferates or dies. Through which effectors might oncoproteins
     generate sensitivity to apoptosis remains to be determined. Ras GTPase
     activating protein (Ras-GAP) is a key element in the Ras signalling
     pathway, being both a negative regulator and possibly an effector of Ras.
     Ras-GAP acts as a regulator of transcription, and possibly connects Ras to
     stress-activated protein kinases. A role for Ras-GAP in cell survival has
     been suspected from the study of knock-out mouse embryos. In search for
     selective killing of tumour cells, we asked whether Ras-GAP inhibition by
```

other means would lead to apoptosis in established cell lines. We injected a **monoclonal antibody** directed against the **SH3** domain of Ras-GAP (mAb200) that has been shown to block Ras-GAP downstream signalling into various human normal and tumour cell lines. We show that inhibition of Ras-GAP induces apoptosis specifically in tumour, but not in normal cells, therefore pointing at a specific role for Ras-GAP in tumour cell survival. MAb200-induced apoptosis is largely prevented by coinjection of activated RhoA or **Cdc42** proteins, by injection of a constitutively activated mutant of phosphoinositide 3-OH kinase (PI3-K), but not by injection of v-Raf. These results show that targeting of Ras-GAP could represent a novel anticancer approach.

L9 ANSWER 2 OF 2 MEDLINE
1998378568 Document Number: 98378568. PubMed ID: 9710640. Ras-GAP controls Rho-mediated cytoskeletal reorganization through its **SH3** domain. Leblanc V; Tocque B; Delumeau I. (Rhone-Poulenc Rorer Central Research, Gene Medicine Department, Centre de Recherche de Vitry Alfortville, 94403 Vitry sur Seine, France.. veronique.leblanc@exonhit.com) . MOLECULAR AND CELLULAR BIOLOGY, (1998 Sep) 18 (9) 5567-78. Journal code: NGY; 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB Proteins of the Ras superfamily, Ras, Rac, Rho, and **Cdc42**, control the remodelling of the cortical actin cytoskeleton following growth factor stimulation. A major regulator of Ras, Ras-GAP, contains several structural motifs, including an **SH3** domain and two SH2 domains, and there is evidence that they harbor a signalling function. We have previously described a **monoclonal antibody** to the **SH3** domain of Ras-GAP which blocks Ras signalling in Xenopus oocytes. We now show that microinjection of this **antibody** into Swiss 3T3 cells prevents the formation of actin stress fibers stimulated by growth factors or activated Ras, but not membrane ruffling. This inhibition is bypassed by coinjection of activated Rho, suggesting that the Ras-GAP **SH3** domain is necessary for endogenous Rho activation. In agreement, the **antibody** blocks lysophosphatidic acid-induced neurite retraction in differentiated PC12 cells. Furthermore, we demonstrate that microinjection of full-length Ras-GAP triggers stress fiber polymerization in fibroblasts in an **SH3**-dependent manner, strongly suggesting an effector function besides its role as a Ras downregulator. These results support the idea that Ras-GAP connects the Ras and Rho pathways and, therefore, regulates the actin cytoskeleton through a mechanism which probably does not involve p190 Rho-GAP.

=> dup remove l5
PROCESSING COMPLETED FOR L5
L10 41 DUP REMOVE L5 (20 DUPLICATES REMOVED)

=> d l10 1-41 cbib abs

L10 ANSWER 1 OF 41 CAPLUS COPYRIGHT 2002 ACS
2001:360176 Document No. 134:362271 Protein and cDNA sequences of novel human and mouse **Cdc42**/Rac interactive binding (CRIB) protein zmse1. Holloway, James L.; Gao, Zeren; Whitmore, Theodore E. (ZymoGenetics, Inc., USA). PCT Int. Appl. WO 2001034803 A2 20010517, 132 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US30945 20001109. PRIORITY: US 1999-438564 19991110.

AB The present invention provides protein and cDNA sequences for a newly identified human and mouse CRIB protein zmse1. The zmse1 gene is mapped on human chromosome 17q24.3-q25. The present invention also includes **antibodies** to the zmse1 polypeptides. Nothern blotting results show that zmse1 mRNA signal is ubiquitous for tissues tested. Expression of zmse1 in cancer tissues using NCI60 cancer microarray suggest that zmse1 has a role in cell cycle control and cancer.

L10 ANSWER 2 OF 41 MEDLINE

2001515589 Document Number: 21234602. PubMed ID: 11329369. Recruitment and activation of Rac1 by the formation of E-cadherin-mediated cell-cell adhesion sites. Nakagawa M; Fukata M; Yamaga M; Itoh N; Kaibuchi K. (Division of Signal Transduction, Nara Institute of Science and Technology, Ikoma 630-0101, Japan.) JOURNAL OF CELL SCIENCE, (2001 May) 114 (Pt 10) 1829-38. Journal code: HNK; 0052457. ISSN: 0021-9533. Pub. country: England: United Kingdom. Language: English.

AB Rac1, a member of the Rho family small GTPases, regulates E-cadherin-mediated cell-cell adhesion. However, it remains to be clarified how the localization and activation of Rac1 are regulated at sites of cell-cell contact. Here, using enhanced green fluorescence protein (EGFP)-tagged Rac1, we demonstrate that EGFP-Rac1 is colocalized with E-cadherin at sites of cell-cell contact and translocates to the cytosol during disruption of E-cadherin-mediated cell-cell adhesion by Ca(2+) chelation. Re-establishment of cell-cell adhesion by restoration of Ca(2)(+) caused EGFP-Rac1 to become relocalized, together with E-cadherin, at sites of cell-cell contact. Engagement of E-cadherin to the apical membrane by anti-E-cadherin **antibody** (ECCD-2) recruited EGFP-Rac1. We also investigated whether E-cadherin-mediated cell-cell adhesion induced Rac1 activation by measuring the amounts of GTP-bound Rac1 based on its specific binding to the **Cdc42**/Rac1 interactive binding region of p21-activated kinase. The formation of E-cadherin-mediated cell-cell adhesion induced Rac1 activation. This activation was inhibited by treatment of cells with a neutralizing **antibody** (DECMA-1) against E-cadherin, or with wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI 3-kinase). IQGAP1, an effector of Rac1, and EGFP-Rac1 behaved in a similar manner during the formation of E-cadherin-mediated cell-cell adhesion. Rac1 activation was also confirmed by measuring the amounts of coimmunoprecipitated Rac1 with IQGAP1 during the establishment of cell-cell adhesion. Taken together, these results suggest that Rac1 is recruited at sites of E-cadherin-mediated cell-cell adhesion and then activated, possibly through PI 3-kinase.

L10 ANSWER 3 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)

2001:340084 The Genuine Article (R) Number: 423YK. Analysis of R-Ras signalling pathways. Self A J; Caron E; Paterson H F; Hall A (Reprint). Univ Coll London, MRC, Mol Cell Biol Lab, CRC Oncogene & Signal Transduct Grp, Gower St, London WC1E 6BT, England (Reprint); Univ Coll London, MRC, Mol Cell Biol Lab, CRC Oncogene & Signal Transduct Grp, London WC1E 6BT, England; Univ Coll London, Dept Biochem, London WC1E 6BT, England; Inst Canc Res, Chester Beatty Labs, CRC, Ctr Cell & Mol Biol, London SW3 6JB, England. JOURNAL OF CELL SCIENCE (APR 2001) Vol. 114, No. 7, pp. 1357-1366 . Publisher: COMPANY OF BIOLOGISTS LTD. BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND. ISSN: 0021-9533. Pub. country: England. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB R-Ras has a high degree of sequence homology to Ras and to other members of the Ras subfamily including Rap, TC21 and ill-Ras. Activated versions of Ras and TC21 are highly transforming in a variety of cell lines and mutated forms of both proteins have been found in human tumours, R-Ras interacts with many of the same proteins as Res and TC21, including c-Raf1, and call induce transformed foci, although this activity is weak compared to Ras and appears to be cell-type specific, Here, we have

investigated R-Ras signalling pathways in a variety of cell types, We find that microinjection of activated R-Ras into quiescent fibroblasts stimulates cell cycle progression through G(1) phase and subsequent DNA synthesis. However, unlike Bas, R-Ras does not activate the ERK MAP kinase pathway nor does it activate the JNK or p38/Mpk2 MAP kinase pathways. microinjection of R-Ras into PC12 cells does not induce terminal differentiation, but instead causes extensive cell spreading, consistent with R-Ras having a role in integrin activation. Finally, in a macrophage cell line, R-Ras activates the alphaM beta (2) integrin ria the small GTPase Rapt, leading to phagocytosis of opsonized red blood cells, whereas Ras does not, These results indicate that R-Ras has an important role in the regulation of cell growth and adhesion, but that this is mediated through downstream signals distinct from those used by Ras.

L10 ANSWER 4 OF 41 MEDLINE

2001653231 Document Number: 21562702. PubMed ID: 11706053. Phosphatidylserine (PS) induces PS receptor-mediated macropinocytosis and promotes clearance of apoptotic cells. Hoffmann P R; deCathelineau A M; Ogden C A; Leverrier Y; Bratton D L; Daleke D L; Ridley A J; Fadok V A; Henson P M. (Program in Cell Biology, Department of Pediatrics, National Jewish Medical and Research Center, Denver, CO 80206, USA.) JOURNAL OF CELL BIOLOGY, (2001 Nov 12) 155 (4) 649-59. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB Efficient phagocytosis of apoptotic cells is important for normal tissue development, homeostasis, and the resolution of inflammation. Although many receptors have been implicated in the clearance of apoptotic cells, the roles of these receptors in the engulfment process have not been well defined. We developed a novel system to distinguish between receptors involved in tethering of apoptotic cells versus those inducing their uptake. Our results suggest that regardless of the receptors engaged on the phagocyte, ingestion does not occur in the absence of phosphatidylserine (PS). Further, recognition of PS was found to be dependent on the presence of the PS receptor (PSR). Both PS and anti-PSR **antibodies** stimulated membrane ruffling, vesicle formation, and "bystander" uptake of cells bound to the surface of the phagocyte. We propose that the phagocytosis of apoptotic cells requires two events: tethering followed by PS-stimulated, PSR-mediated macropinocytosis.

L10 ANSWER 5 OF 41 MEDLINE

2001543962 Document Number: 21474428. PubMed ID: 11590197. Differential regulation of transendothelial migration of THP-1 cells by ICAM-1/LFA-1 and VCAM-1/VLA-4. Ronald J A; Ionescu C V; Rogers K A; Sandig M. (Department of Anatomy and Cell Biology, The University of Western Ontario, London, Ontario, Canada.) JOURNAL OF LEUKOCYTE BIOLOGY, (2001 Oct) 70 (4) 601-9. Journal code: IWY; 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

AB The adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expressed in atherogenic lesions are thought to regulate monocyte diapedesis. To better understand their specific roles we used function-blocking **antibodies** and examined in a culture model the morphology, motility, and diapedesis of THP-1 cells interacting with human coronary artery endothelial cells. The number of motile THP-1 cells was reduced only when VCAM-1 or both ICAM-1 and VCAM-1 were blocked. Blockade of ICAM-1 and VCAM-1, either separately or together, reduced to the same degree the distance that THP-1 cells traveled. Diapedesis was reduced only during the simultaneous blockade of both adhesion molecules. Blockade of either ICAM-1 or VCAM-1 inhibited pseudopodia formation, but ICAM-1 blockade induced the formation of filopodia. We suggest that the interactions of endothelial ICAM-1 and VCAM-1 with their ligands differentially regulate distinct steps of diapedesis by modulating the ratio of active and inactive forms of small GTPases such as Rho, Rac, and **Cdc42**.

L10 ANSWER 6 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:278506 The Genuine Article (R) Number: 416CL. Loss of alpha 3 beta 1 integrin function results in an altered differentiation program in the mouse submandibular gland. Menko K S (Reprint); Kreidberg J A; Ryan T T; Van Bockstaele E; Kukuruzinska M A. Thomas Jefferson Univ, Dept Pathol Anat & Cell Biol, 571 Jefferson Alumni Hall, 1020 Locust St, Philadelphia, PA 19107 USA (Reprint); Thomas Jefferson Univ, Dept Pathol Anat & Cell Biol, Philadelphia, PA 19107 USA; Harvard Univ, Sch Med, Dept Pediat, Boston, MA 02115 USA; Childrens Hosp, Dept Med, Boston, MA 02115 USA; Boston Univ, Sch Med, Dept Biochem, Boston, MA 02118 USA; Boston Univ, Sch Dent Med, Dept Mol & Cell Biol, Boston, MA 02118 USA. DEVELOPMENTAL DYNAMICS (APR 2001) Vol. 220, No. 4, pp. 337-349. Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA. ISSN: 1058-8388. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Mammalian submandibular gland (SMG) development leads to the establishment of highly organized secretory acinar and nonsecretory ductal epithelial cells. The ability of maturing salivary epithelial cells to attain their differentiated state has been shown to depend, in part, on interactions between extracellular matrix (ECM) proteins and their integrin receptors. In a search for key regulators of salivary cell lineage, we have studied alpha3 beta1 integrin, a receptor for the basement membrane protein laminin, by characterizing embryonic day 18 (E18) SMGs isolated from mice carrying a targeted mutation in the alpha3 integrin gene. Transmission electron microscopy studies showed that the mutant SMGs exhibited an aberrant differentiation phenotype with defects in the apical-basal polarity axis and in the basement membrane. Based on immunohistochemistry and Western blot analyses, the alpha3 beta1-deficient SMGs had altered expression and/or localization of several ECM and adhesive molecules, including laminin beta1, fibronectin, alpha5 integrin, and E-cadherin, These changes correlated with alterations in the activation state of Ras-extracellular signal-regulated kinase (ERK), as well as the expression and/or localization of **Cdc42** and RhoA, two Rho GTPases that regulate the organization of the actin cytoskeleton. We conclude that alpha3 beta1 is required for normal salivary cell differentiation and that its absence affects multiple components of adhesive complexes and their associated signalling pathways. (C) 2001 Wiley-Liss, Inc.

L10 ANSWER 7 OF 41 MEDLINE
2001235080 Document Number: 21126697. PubMed ID: 11223464. Biochemical and morphological analysis on the localization of Rac1 in neurons. Kumanogoh H; Miyata S; Sokawa Y; Maekawa S. (Department of Biotechnology, Faculty of Textile Science, Kyoto Institute of Technology, 606-8585, Kyoto, Japan.) NEUROSCIENCE RESEARCH, (2001 Feb) 39 (2) 189-96. Journal code: OAQ; 8500749. ISSN: 0168-0102. Pub. country: Ireland. Language: English.

AB The acquisition of cell type-specific morphologies is a central feature of neuronal differentiation. Many extra- and intracellular signals are known to cause the morphological changes of neuronal cells through the reconstruction of the microfilaments underneath the cell membrane. The membrane microdomain called "raft" has been paid much attention, for this domain contains many signal-transducing molecules including trimeric G proteins and cytoskeletal proteins. The raft domain is recovered in a low-density fraction after the treatment of the membrane with the non-ionic detergent such as Triton X-100 and the enrichment of cholesterol and sphingolipids is ascribed to be responsible for the detergent insolubility. In contrast to the well-known localization of trimeric G proteins in raft, the localization of small G proteins in the raft is poorly characterized. Since Rho family small G proteins (Rho, Rac, and **Cdc42**) regulate the microfilament system, we studied the localization of Rho family small G proteins in the raft of rat brain with western blotting. Specific localization of Rac1 was detected in the raft

from 10-day-old and 8-week-old rat whole brain, and also in the raft prepared from the growth cone and synaptic plasma membrane fractions. Rho and **Cdc42** were, in contrast, recovered in the Triton soluble fraction. Double immunostaining of cultured hippocampal neurons with **antibodies** to Rac1 and MAP-2, or Rac1 and tau, showed punctate distribution of Rac1 in axons as well as in dendrites.

L10 ANSWER 8 OF 41 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:153051 Document No.: PREV200200153051. **Cdc42**, Rac1, and Rac2 expression and GTP-binding in normal and chronic myelogenous leukemia (CML) CD34+ hematopoietic progenitor cells (HPC. Brunstein, Claudio G. (1); Verfaillie, Catherine M. (1). (1) Medicine, University of Minnesota, Minneapolis, MN USA. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 142a. http://www.bloodjournal.org/. print. Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001 ISSN: 0006-4971. Language: English.

AB We and others have shown that the BCR/ABL fusion protein product, a constitutionally active tyrosine kinase, interferes with normal beta-1-integrin signaling pathways leading to decreased adhesion and increased migration of CML cells. The RhoGTPases **Cdc42** and Rac regulate cytoskeleton remodeling, adhesion, and migration of murine hematopoietic stem cells, and also have been implicated in the control of proliferation and gene expression. Elimination of Rac2, the predominant form of Rac in hematopoietic cells, causes decreased migration of HPC in mouse and decreased phagocyte function and activation in humans. We studied the expression and GTP-loading of **Cdc42**, Rac1, and Rac2 in Bcr/Abl positive and negative hematopoietic cells lines, and in human normal and CML derived CD34-positive cells. For the western-blots we used mouse **monoclonal** anti-**Cdc42** and anti-Rac1 (BD Transduction Laboratories, Lexington, KY) and rabbit polyclonal anti-Rac2 (with a kind gift from Dr. G. Bokoch, The Scripps Research Institute). The pull-down assay was performed with the commercially available GST fusion-protein, corresponding to the p21-binding domain of human PAK-1 (**Cdc42**/Rac Activation Assays Kit, Upstate Biotechnology, Lake Placid, NY). In human mononuclear and CD34+ HPC from normal donors and CML patients in chronic phase the levels of **Cdc42**, Rac1, and Rac2 protein were similar. CML peripheral blood mononuclear cells contained increased active GTP bound **Cdc42** and decreased amounts of active Rac1 and Rac2 when compared to normal cells. Whereas increased active, GTP-loaded **Cdc42** was present in CML CD34+ cells compared to normal CD34+ HPC, the decrease in active, GTP-bound Rac1 and Rac2 was less prominent. Upon engagement of beta-1-integrins with a blocking **monoclonal antibody**, GTP loading of **Cdc42**, Rac1 and Rac2 increased in CD34+ HPC. Unexpectedly, the activation of **Cdc42**, Rac1 and Rac2 decreased in CML HPC following integrin engagement. Treatment of CML CD34+ HPC with 1 micromolar of the tyrosine kinase inhibitor STI571 (Novartis, Basel, Switzerland) overnight, restored "normal" activation of GTPases in the absence of integrin activation (i.e. lowered GTP-bound protein), and upregulation of GTP loading following beta-1-integrin engagement in CML CD34+ HPC. These studies show that (1) presence of Bcr/Abl leads to spontaneous activation of the GTPase **Cdc42**, but downregulates Rac1, and Rac2 GTP loading, which may contribute to the abnormal spontaneous migration and the abnormal baseline skeletal activation in CML CD34+ HPC. (2) In CML CD34+ HPC, GTPase activation seems to be decreased following integrin engagement, which may contribute to the decreased adhesion to and migration over fibronectin. (3) As these phenomena are blocked by STI571, the effect of Bcr/Abl is mediated by its tyrosine kinase fucntion. Whether inhibition of GTPase activitiy will restore normal hematopoietic cell function in CML is currently being studied.

L10 ANSWER 9 OF 41 MEDLINE
2001357832 Document Number: 21311862. PubMed ID: 11418640.

Antibody-induced shedding of CD44 from adherent cells is linked to the assembly of the cytoskeleton. Shi M; Dennis K; Peschon J J; Chandrasekaran R; Mikecz K. (Departments of. Biochemistry and Orthopedic Surgery, Section of Biochemistry and Molecular Biology, Rush University at Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612, USA.) JOURNAL OF IMMUNOLOGY, (2001 Jul 1) 167 (1) 123-31. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB CD44 is a widely expressed integral membrane glycoprotein that serves as a specific adhesion receptor for the extracellular matrix glycosaminoglycan hyaluronan. CD44 participates in a variety of physiological and pathological processes through its role in cell adhesion. Under appropriate conditions, the ectodomain of CD44 is proteolytically removed from the cell surface. In this study we show that excessive CD44 shedding can be induced in mouse fibroblasts and monocytes upon exposure of these cells to a CD44-specific Ab immobilized on plastic, whereas treatment with phorbol ester induces significantly enhanced CD44 release from the monocytes only. CD44 shedding proceeds normally in fibroblasts and monocytes deficient in TNF-alpha converting enzyme (TACE), a sheddase involved in the processing of several substrates. Conversely, activation of the CD44 protease has no effect on the release of TNF-alpha from TACE-expressing cells, although the same metalloprotease inhibitor effectively blocks both TACE and the CD44 sheddase. Concomitant with anti-CD44 Ab- or phorbol ester-induced CD44 shedding, dramatic changes are observed in cell morphology and the structure of the actin cytoskeleton. Disruption of actin assembly with cytochalasin reduces CD44 shedding, but not the release of TNF-alpha. Moreover, pharmacological activation of Rho family GTPases Rac1 and **Cdc42**, which regulate actin filament assembly into distinct cytoskeletal structures, has a profound effect on CD44 release. We conclude that the CD44 sheddase and TACE are distinct enzymes, and that Ab- and phorbol ester-enhanced cleavage of CD44 is controlled in a cell type-dependent fashion by Rho GTPases through the cytoskeleton.

L10 ANSWER 10 OF 41 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

2001068248 EMBASE A new home for infection research. Kamradt T.; Rudel T.; Selbach M.; Schaible U.E.. U.E. Schaible, Max-Planck-Inst. for Infect. Biology, German Ctr. for Rheumatological Res., Schumannstr. 21-22, D-10117 Berlin, Germany. schaible@mpiib-berlin.mpg.de. Trends in Microbiology 9/2 (54-56) 1 Feb 2001.
ISSN: 0966-842X. CODEN: TRMIEA.
Publisher Ident.: S 0966-842X(00)01938-7. Pub. Country: United Kingdom.
Language: English. Summary Language: English.

AB The inaugural symposium of the Max-Planck-Institute for Infection Biology and German Center for Rheumatological Research was held in Berlin, 29 September 2000.

L10 ANSWER 11 OF 41 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2002:129516 Document No.: PREV200200129516. Interaction between SHPS-1 and CD47 mediates the adhesion of human B lymphocytes to non-activated endothelial cells. Yoshida, Hitoshi (1); Tomiyama, Yoshiaki (1); Oritani, Kenji (1); Honma, Nakayuki; Matsuzawa, Yuji (1). (1) Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Suita, Osaka Japan. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 21a. http://www.bloodjournal.org/. print. Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001 ISSN: 0006-4971. Language: English.

AB CD47, also known as integrin-associated protein, is an ubiquitously exressed 50-kd cell surface glycoprotein with an extracellular immunoglobulin domain and 5 putative transmembrane domains. It physically and functionally associates with beta 3 integrins and modulates a variety of cell functions including cell activation, adhesion, migration, and phagocytosis. Treatment of leukocytes with anti-CD47 **monoclonal antibodies** (mAbs) modulates beta3 integrin-mediated ligand

binding, activation, oxidative burst, and Fc receptor-mediated phagocytosis. Neutrophils require CD47 to migrate across the endothelial and colonic epithelial cells after firm adhesion. We have recently demonstrated that soluble form of an anti-CD47 mAb B6H12 induces polarization in these B cell lines via the activation of **Cdc42**, a member of Rho family small GTPase in an integrin-independent manner. These findings suggest that CD47 itself may transduce polarization signals into B lymphocytes. Because these studies have been conducted by using some ligand-mimic anti-CD47 mAbs, the roles of interactions between CD47 and its ligands thrombospondin (TSP) and SHPS-1, still remain elusive. Employing a fusion protein consisted of the extracellular region of SHPS-1 and the Fc portion of human immunoglobulin (SHPS-1-Ig), we investigated the effects of SHPS-1 as a ligand for CD47 on B lymphocytes. Although SHPS-1-Ig binding to human B cell lines was solely mediated via CD47, their binding capacity for soluble and immobilized SHPS-1-Ig varied among cell lines irrespective of the similar expression levels of CD47, suggesting that distinctive affinity/avidity states exist during B cell maturation. Nalm6 cell line and tonsilar B lymphocytes adhered to immobilized SHPS-1-Ig and showed polarization-like morphology. These effects of SHPS-1-Ig were blocked by anti-CD47 mAbs (B6H12 and SE5A5) but not 4N1K, a functional peptide of thrombospondin (TSP). Wortmannin, a phosphatidyl inotitol-3 kinase inhibitor, but not pertussis toxin significantly inhibited the polarization induced by the immobilized SHPS-1-Ig. Thus, SHPS-1 acts as an adhesive substrate via CD47 in human B lymphocyte, and the SHPS-1 binding site in CD47 is probably different from the TSP binding site. Immunohistochemical analyses indicated that SHPS-1 is expressed on high endothelial venule as well as macrophages in human tonsils. Human umbilical vein endothelial cells (HUVECs) also express SHPS-1 in the absence of any stimuli, and the adhesion of tonsilar B lymphocytes to non-activated HUVECs was significantly inhibited By SE5A5, indicating that SHPS 1/CD47 interaction is involved in the adhesion. Our findings suggest that SHPS-1/CD47 interaction may contribute to the recruitment of B lymphocytes via endothelial cells under steady state conditions.

L10 ANSWER 12 OF 41 CAPLUS COPYRIGHT 2002 ACS

2000:756864 Document No. 133:318809 Signal transduction protein 19G5 and cDNA and their use in treatment of cardiac disease. Zeng, Wenlin; Stanton, Lawrence; Kong, Haiyan (Scios, Inc., USA). PCT Int. Appl. WO 2000063381 A1 20001026, 82 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9488 20000411. PRIORITY: US 1999-PV129553 19990416.

AB Proteins capable of regulating signal transduction, which preferably exhibit kinase activity, or **antibodies** against such proteins that inhibit the interaction of these proteins with other mediators of signal transduction, may be used in the identification, prevention, or treatment of disease, preferably cardiac disease, in mammalian hosts. In addn., these proteins can facilitate the identification or isolation of addnl. mediators of signal transduction assocd. with disease, preferably cardiac disease, which in turn may also be used in the identification, prevention, or treatment of disease, preferably cardiac disease, in mammals. Thus, three human cDNAs for 1610-, 2596-, and 871-amino acid splice variants of a protein encoded by gene 19G5 expressed in heart tissue are disclosed. Rat and mouse cDNAs were also cloned and sequenced. The 2596-amino acid isoform contained kinase domains. In addn. this isoform exhibited binding to **Cdc42**. The gene was strongly

expressed in heart tissue. Gene 19G5 appears to be present in the human gene as a single copy. The expression of this 19G5 was significantly increased during differentiation of myoblasts into myotubes.

L10 ANSWER 13 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:528201 The Genuine Article (R) Number: 331XB. Rac1 GTPases control filopodia formation, cell motility, endocytosis, cytokinesis and development in Dictyostelium. Dumontier M; Hocht P; Mintert U; Faix J (Reprint). UNIV WISCONSIN, DEPT PHYSIOL, 1300 UNIV AVE, MADISON, WI 53706 (Reprint); MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED, GERMANY. JOURNAL OF CELL SCIENCE (JUN 2000) Vol. 113, No. 12, pp. 2253-2265. Publisher: COMPANY OF BIOLOGISTS LTD. BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND. ISSN: 0021-9533. Pub. country: USA; GERMANY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The function of the highly homologous Rac1A, Rac1B, and Rac1C GTPases of the Dictyostelium Rad group was investigated. All three GTPases bound with an equal capacity to the IQGAP-related protein DGAP1, with a preference for the activated GTP-bound form. Strong overexpression of wild-type Rad GTPases N-terminally tagged with green fluorescent protein (GFP), predominantly induced the formation of numerous long filopodia, Remarkably, expression of the constitutively-activated GTPases resulted in dominant-negative phenotypes: these Rac1-V12 mutants completely lacked filopodia but formed numerous crown shaped structures resembling macropinosomes. Moreover, these mutants were severely impaired in cell motility, colony growth, phagocytosis, pinocytosis, cytokinesis and development. Transformants expressing constitutively-inactivated Rac1-N17 proteins were similar to wild-type cells, but displayed abundant and short filopodia and exhibited a moderate defect in cytokinesis. Taken together, our results indicate that the three GTPases play an identical role in signaling pathways and are key regulators of cellular activities that depend on the re-organization of the actin cytoskeleton in Dictyostelium.

L10 ANSWER 14 OF 41 CAPLUS COPYRIGHT 2002 ACS
2000:656187 Document No. 134:142563 Characterization of a **monoclonal antibody** panel shows that the myotonic dystrophy protein kinase, DMPK, is expressed almost exclusively in muscle and heart. Lam, L. T.; Pham, Y. C. N.; Man, Nguyen Thi; Morris, G. E. (MRIC Biochemistry Group, North East Wales Institute, Wrexham, LL11 2AW, UK). Human Molecular Genetics, 9(14), 2167-2173 (English) 2000. CODEN: HMGEE5. ISSN: 0964-6906. Publisher: Oxford University Press.
AB Myotonic dystrophy (DM) is a multisystemic disorder caused by an inherited CTG repeat expansion which affects three genes encoding the DM protein kinase (DMPK), a homeobox protein Six5 and a protein contg. WD repeats. Using a panel of 16 **monoclonal antibodies** against several different DMPK epitopes we detected DMPK, as a single protein of .apprx.80 kDa, only in skeletal muscle, cardiac muscle and, to a lesser extent, smooth muscle. Many earlier reports of DMPK with different sizes and tissue distributions appear to be due to **antibody** cross-reactions with more abundant proteins. One such **antibody**, MANDM1, was used to isolate two related protein kinases, MRCK.alpha. and .beta., from a human brain cDNA library and the shared epitope was located at the catalytic site of DMPK using a phage-displayed random peptide library. The peptide library also identified an epitope shared between DMPK and a 55 kDa muscle-specific protein. The results suggest that effects of the repeat expansion on the DMPK gene may be responsible for muscle and heart features of DM, whereas clin. changes in other tissues may be due to effects on the other two genes.

L10 ANSWER 15 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:262882 The Genuine Article (R) Number: 298QN. Modulating fibroblast adhesion, spreading, and proliferation using self-assembled monolayer films of alkylthiolates on gold. McClary K B; Ugarova T; Grainger D W

(Reprint). COLORADO STATE UNIV, DEPT BIOCHEM, FT COLLINS, CO 80523 (Reprint); COLORADO STATE UNIV, DEPT BIOCHEM, FT COLLINS, CO 80523; COLORADO STATE UNIV, DEPT CHEM, FT COLLINS, CO 80523; CLEVELAND CLIN FDN, DEPT MOL CARDIOL, CTR THROMBOSIS & VASC BIOL, CLEVELAND, OH 44195. JOURNAL OF BIOMEDICAL MATERIALS RESEARCH (5 JUN 2000) Vol. 50, No. 3, pp. 428-439. Publisher: JOHN WILEY & SONS INC. 605 THIRD AVE, NEW YORK, NY 10158-0012. ISSN: 0021-9304. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Ultrathin, highly organized functionalized alkylthiol monolayers were applied as model substrates for cell growth and protein adsorption studies. The aim of this approach was to improve the understanding of molecular surface determinants required for adhesion-dependent cell growth and proliferation using well-controlled surface chemistry. Carboxyl- and methyl-terminated alkylthiol monolayers on gold were used to monitor Swiss 3T3 fibroblast adhesion, spreading, and growth. Stress fiber and focal contact formation were determined by immunostaining of actin filaments and paxillin. Fibronectin deposition and conformation on these surface chemistries in the presence and absence of competing proteins were also determined. The relative levels of adsorbed fibronectin were assessed using radiolabeled proteins Exposure of the 10th type III cell integrin binding domain of fibronectin was assessed using a radiolabeled **monoclonal antibody**. Distinct alkylthiol substrate chemistry-dependent differences were observed in fibroblast adhesion, spreading, and growth. The formation of focal contacts and stress fibers was enhanced on the carboxyl-terminated surface relative to the methyl surface. Relative deposition and conformations of adsorbed fibronectin were shown to he dependent on surface chemistry in both the presence and absence of competing proteins. The results indicated that well-controlled culture surfaces modulate differential cell adhesion, spreading, and growth through modulations of the amounts and conformations of adsorbed extracellular matrix molecules (e.g., fibronectin). (C) 2000 John Wiley & Sons, Inc.

L10 ANSWER 16 OF 41 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:311546 Document No.: PREV200100311546. Characterization of the Rho-family GTPase activating protein IQGAP2 in platelet activation. Cupit, Lisa D. (1); Schmidt, Valentina (1); Wainer, Jean (1); Scudder, Lesley (1); Bahou, Wadie F. (1). (1) Department of Medicine, State University of New York, Stony Brook, NY USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 243a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology. ISSN: 0006-4971. Language: English. Summary Language: English.

AB Proteolytically activated receptors (PARs) are G protein-coupled seven transmembrane receptors that transmit regulatory intracellular signals via activation of proteases generated during hemostatic, inflammatory or fibrinolytic pathways. Previous genomic characterization from this laboratory using radiation hybrid and physical mapping techniques has identified an apprx150 kb PAR gene cluster at 5q13, ordered centromere-D5S424-PAR2-PAR1-PAR3-D5S2529-telomere. Genomic scanning identified the putative Rho-family GTPase activating protein IQGAP2 as the single gene flanked by the thrombin receptors PAR1 and PAR3, suggesting its function as a genomic unit involved in thrombin signaling. Cell- or tissue-specific transcript expression was evident in human erythroleukemia (HEL) and HepG2 (liver) cells, spleen, and platelets using IQGAP2-specific oligonucleotide primers and reverse transcriptase-PCR, and immunoblot analysis established the presence of the apprx180 kDa protein in whole platelet lysates. Unstimulated, permeabilized platelets demonstrated a relatively uniform pattern of IQGAP2 expression as visualized by immunofluorescence staining. Since the rho-family GTPases rac1 and/or **CDC42** have been identified as intermediaries for thrombin-induced actin filament uncapping, the functional relationship between IQGAP2 and these rho-GTPases was subsequently studied. Preliminary data using

thrombin (0.1 U/mL, 1 nM)- or TRAP42-47 (20 muM)-stimulated gel-filtered platelets demonstrated rapid increases in cytoplasmic IQGAP2, displaying ligand-distinctive differences over a time range of 15-60 seconds following platelet stimulation. Furthermore, immunoprecipitation using **monoclonal antibodies** to IQGAP2, rac1, **CDC42** and platelet glycoprotein IIb/IIIa demonstrated that rac1- and GPIIb/IIIa-immunoprecipitates specifically bound IQGAP2 as demonstrated by subsequent SDS-PAGE and immunoblot analysis. In contrast, there was little to no evidence for formation of a **CDC42**/IQGAP2 complex, suggesting a restricted interaction between these rho-family GTPases. Thus our data suggest the evolution of a functional genomic unit within the PAR gene cluster, and implicate IQGAP2 in a unique signaling pathway, functioning as a modulator or effector protein in thrombin-mediated activation pathways and cytoskeletal actin reorganization in humans.

L10 ANSWER 17 OF 41 MEDLINE DUPLICATE 1
2000384086 Document Number: 20352006. PubMed ID: 10891456.
Integrin-associated protein/CD47 regulates motile activity in human B-cell lines through **CDC42**. Yoshida H; Tomiyama Y; Ishikawa J; Oritani K; Matsumura I; Shiraga M; Yokota T; Okajima Y; Ogawa M; Miyagawa J i; Nishiura T; Matsuzawa Y. (Department of Internal Medicine and Molecular Science and the Department of Hematology and Oncology, Osaka University, Suita, Japan.) BLOOD, (2000 Jul 1) 96 (1) 234-41. Journal code: A8G; 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.
AB Cell migration requires a dynamic interaction between the cell, its substrate, and the cytoskeleton-associated motile apparatus. Integrin-associated protein (IAP)/CD47 is a 50-kd cell surface protein that is physically associated with beta3 integrins and that modulates the functions of beta3 integrins in various cells. However, in B-lymphocytes that express beta1 integrins but few beta3 integrins, the roles of IAP/CD47 remain to be determined. Cross-linking of IAP/CD47 by the immobilized anti-IAP/CD47 **monoclonal antibody** (mAb) B6H12, but not 2D3, produced signals to promote polarization with lamellipodia, a characteristic morphology during leukocyte migration, in pre-B and mature B-cell lines (BALL, Nalm6, ONHL-1, Daudi), but not in myeloma cell lines (RPMI8226, OPM-2). In the presence of the immobilized fibronectin (FN), soluble B6H12 could increase the rate of the polarization and activate migratory activity of BALL cells to FN in a transwell filter assay. Furthermore, the dominant-negative form of **CDC42** completely blocked B6H12-induced morphologic and functional changes without inhibiting phorbol 12-myristate 13-acetate-induced spreading on FN in BALL cells, whereas the dominant-negative form of Rac1 inhibited all these changes. These findings demonstrate that in B-lymphocytes, IAP/CD47 may transduce the signals to activate the migratory activity, in which **CDC42** may be specifically involved, and that IAP/CD47 shows synergistic effect with alpha4beta1 on B-cell migration. These findings would provide new insight into the role of IAP/CD47 on B-cell function.

L10 ANSWER 18 OF 41 CAPLUS COPYRIGHT 2002 ACS
1999:784251 Document No. 132:19663 human Pak4 novel gene encoding a serine/threonine kinase useful as tumor cell inhibitor and active in induction of filopodia and actin cytoskeleton polymerization. Minden, Audrey (The Trustees of Columbia University In the City of New York, USA). PCT Int. Appl. WO 9963073 A1 19991209, 96 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US11341 19990521. PRIORITY: US 1998-82737 19980521.

AB This invention provides an isolated mammalian nucleic acid mol. encoding a PAK4 serine/threonine kinase. This invention provides an isolated nucleic acid mol. encoding a mutant homolog of the mammalian PAK4 serine/threonine kinase whose amino acid sequence is set forth. This invention provides a fusion protein comprising a PAK4 serine/threonine kinase or a fragment thereof and a second peptide. This invention provides a purified mammalian PAK4 serine/threonine kinase. This invention provides a protein comprising substantially the amino acid sequence set forth in Figure 1A. This invention provides a **monoclonal antibody** directed to an epitope of a PAK4 serine/threonine kinase. This invention provides a method of inhibiting PAK4 function comprising administering a ligand comprising an amino acid domain which binds to a GTP binding protein so as to inhibit binding of the GTP binding protein to PAK4. This invention provides a method of inhibiting PAK4 function comprising administering a ligand which binds to the GTP binding domain of PAK4 so as to inhibit PAK4 binding to a GTP binding protein. This invention provides a method of inhibiting PAK4 serine/threonine kinase function comprising administering a ligand which blocks an ATP binding domain so as to inhibit PAK4 serine/threonine kinase function. This invention provides a method of inhibiting growth of a tumor cell comprising blocking Cdc42Hs by administering a ligand capable of binding to a Cdc42Hs binding site of a PAK4 serine/threonine kinase. PAK4 was shown to interact with activated Cdc42Hs through GBD/CRIB domain and is recruited to the Golgi. PAK4 is involved with the actin cytoskeleton and activation of the JNK pathway. PAK4 induces actin polymn. and induces formation of filopodia. PAK4 is used as a tumor cell inhibitor for cancer or arthritis. Mouse cDNA and protein fragments are also listed..

L10 ANSWER 19 OF 41 MEDLINE DUPLICATE 2

1999422020 Document Number: 99422020. PubMed ID: 10490822. Ras-GTPase activating protein inhibition specifically induces apoptosis of tumour cells. Leblanc V; Delumeau I; Tocque B. (ExonHit Therapeutics, 65 Bld Massena, 75013 Paris, France.) ONCOGENE, (1999 Aug 26) 18 (34) 4884-9. Journal code: ONC; 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Oncogenes and tumour suppressor genes control the balance between apoptotic death and anti-apoptotic survival signals determining whether a cell proliferates or dies. Through which effectors might oncoproteins generate sensitivity to apoptosis remains to be determined. Ras GTPase activating protein (Ras-GAP) is a key element in the Ras signalling pathway, being both a negative regulator and possibly an effector of Ras. Ras-GAP acts as a regulator of transcription, and possibly connects Ras to stress-activated protein kinases. A role for Ras-GAP in cell survival has been suspected from the study of knock-out mouse embryos. In search for selective killing of tumour cells, we asked whether Ras-GAP inhibition by other means would lead to apoptosis in established cell lines. We injected a **monoclonal antibody** directed against the SH3 domain of Ras-GAP (mAb200) that has been shown to block Ras-GAP downstream signalling into various human normal and tumour cell lines. We show that inhibition of Ras-GAP induces apoptosis specifically in tumour, but not in normal cells, therefore pointing at a specific role for Ras-GAP in tumour cell survival. MAb200-induced apoptosis is largely prevented by coinjection of activated RhoA or **Cdc42** proteins, by injection of a constitutively activated mutant of phosphoinositide 3-OH kinase (PI3-K), but not by injection of v-Raf. These results show that targeting of Ras-GAP could represent a novel anticancer approach.

L10 ANSWER 20 OF 41 MEDLINE DUPLICATE 3

1999298178 Document Number: 99298178. PubMed ID: 10369666. IpaC induces actin polymerization and filopodia formation during Shigella entry into epithelial cells. Tran Van Nhieu G; Caron E; Hall A; Sansonetti P J. (Unite de Pathogenie Microbienne Moleculaire INSERM U389, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France..

gtranvan@pasteur.fr) . EMBO JOURNAL, (1999 Jun 15) 18 (12) 3249-62. Journal code: EMB; 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Shigella proteins that are targeted to host cells by a type III secretion apparatus are essential for reorganization of the cytoskeleton during cell invasion. We have developed a semi-permeabilized cell assay that tests the effects of bacterial proteins on the actin cytoskeleton. The Shigella IpaC protein was found to induce the formation of filopodial and lamellipodial extensions in these semi-permeabilized cells. Microinjection of IpaC into cells, or cellular expression of IpaC also led to the formation of filopodial structures. **Monoclonal antibodies** (mAbs) directed against the C-terminus of IpaC inhibited the IpaC-induced extensions, whereas an anti-N-terminal IpaC mAb stimulated extensive lamellae formation. Shigella induced foci of actin polymerization in the permeabilized cells and these were inhibited by anti-C-terminal IpaC mAbs. Consistent with a role for IpaC in Shigella-induced cytoskeletal rearrangements during entry, stable transfectants expressing IpaC challenged with Shigella showed increased bacterial internalization. IpaC-induced extensions were inhibited by a dominant-interfering form of **Cdc42** or the **Cdc42**-binding domain of WASP, whereas a dominant-interfering form of Rac resulted in inhibition of lamellae formation. We conclude that IpaC leads to activation of **Cdc42** which in turn, causes activation of Rac, both GTPases being required for Shigella entry.

L10 ANSWER 21 OF 41 SCISEARCH · COPYRIGHT 2002 ISI (R)

2000:3652 The Genuine Article (R) Number: 266VM. Induction of cell scattering by expression of beta 1 integrins in beta 1-deficient epithelial cells requires activation of members of the Rho family of GTPases and downregulation of cadherin and catenin function. Gimond C; vanderFlier A; vanDelft S; Brakebusch C; Kuikman I; Collard J G; Fassler R; Sonnenberg A (Reprint). NETHERLANDS CANC INST, DIV CELL BIOL, PLESMANLAAN 121, NL-1066 CX AMSTERDAM, NETHERLANDS (Reprint); NETHERLANDS CANC INST, DIV CELL BIOL, NL-1066 CX AMSTERDAM, NETHERLANDS; UNIV LUND HOSP, EXPT PATHOL SECT, S-22185 LUND, SWEDEN. JOURNAL OF CELL BIOLOGY (13 DEC 1999) Vol. 147, No. 6, pp. 1325-1340. Publisher: ROCKEFELLER UNIV PRESS. 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021. ISSN: 0021-9525. Pub. country: NETHERLANDS; SWEDEN . Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Adhesion receptors, which connect cells to each other and to the surrounding extracellular matrix (ECM), play a crucial role in the control of tissue structure and of morphogenesis. In this work, we have studied how intercellular adhesion molecules and beta 1 integrins influence each other using two different beta 1-null cell lines, epithelial GE11 and fibroblast-like GD25 cells. Expression of beta 1A or the cytoplasmic splice variant beta 1D, induced the disruption of intercellular adherens junctions and cell scattering in both GE11 and GD25 cells. In GE11 cells, the morphological change correlated with the redistribution of zonula occluden (ZO)-1 from tight junctions to adherens junctions at high cell confluency. In addition? the expression of pi integrins caused a dramatic reorganization of the actin cytoskeleton and of focal contacts. Interaction of beta 1 integrins,with their respective ligands was required for a complete morphological transition towards the spindle-shaped fibroblast-like phenotype. The expression of an interleukin-2 receptor (IL2R)-beta 1A chimera and its incorporation into focal adhesions also induced the disruption of cadherin-based adhesions and the reorganization of ECM-cell contacts, but failed to promote cell migration on fibronectin, in contrast to full-length beta 1A, This indicates that the disruption of cell-cell adhesion is not simply the consequence of the stimulated cell migration. Expression of beta 1 integrins in GE11 cells resulted in a decrease in cadherin and alpha-catenin protein levels accompanied by their redistribution from the cytoskeleton-associated fraction to the detergent-soluble fraction, Regulation of alpha-catenin protein levels by

beta 1 integrins is likely to play a role in the morphological transition, since overexpression of alpha-catenin in GE11 cells before beta 1 prevented the disruption of intercellular adhesions and cell scattering. In addition: using biochemical activity assays for Rho-like GTPases, we show that the expression of beta 1A, beta 1D, or IL2R-beta 1A in GE11 or GD25 cells triggers activation of both RhoA and Rac1, but not of **Cdc42**. Moreover, dominant negative Rad (N17Rac1) inhibited the disruption of cell-cell adhesions when expressed before beta 1. However, all three GTPases might be involved in the morphological transition, since expression of either N19RhoA, N17Rac1 or N17Cdc42 reversed cell scattering and partially restored cadherin-based adhesions in GE11-beta 1A cells. Our results indicate that beta 1 integrins regulate the polarity and motility of epithelial cells by the induction of intracellular molecular events involving a downregulation of alpha-catenin function and the activation of the Rho-like G proteins Rac1 and RhoA.

L10 ANSWER 22 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:297204 The Genuine Article (R) Number: 185UK. RacF1, a novel member of the Rho protein family in Dictyostelium discoideum, associates transiently with cell contact areas, macropinosomes, and phagosomes. Rivero F; Albrecht R; Dislich H; Bracco E; Graciotti L; Bozzaro S; Noegel A A (Reprint). UNIV COLOGNE, FAK MED, INST BIOCHEM 1, D-50931 COLOGNE, GERMANY (Reprint); UNIV COLOGNE, FAK MED, INST BIOCHEM 1, D-50931 COLOGNE, GERMANY; MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED, GERMANY; OSPED S LUIGI GONZAGA, DIPARTIMENTO SCI CLIN & BIOL, I-10043 TURIN, ITALY. MOLECULAR BIOLOGY OF THE CELL (APR 1999) Vol. 10, No. 4, pp. 1205-1219. Publisher: AMER SOC CELL BIOLOGY. PUBL OFFICE, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 1059-1524. Pub. country: GERMANY; ITALY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Using a PCR approach we have isolated racF1, a novel member of the Rho family in Dictyostelium. The racF1 gene encodes a protein of 193 amino acids and is constitutively expressed throughout the Dictyostelium life cycle. Highest identity (94%) was found to a RacF2 isoform, to Dictyostelium Rac1A, Rac1B, and Rac1C (70%), and to Rac proteins of animal species (64 - 69%). To investigate the role of RacF1 in cyto skeleton-dependent processes, we have fused it at its amino-terminus with green fluorescent protein (GFP) and studied the dynamics of subcellular redistribution using a confocal laser scanning microscope and a double-view microscope system. GFP-RacF1 was homogeneously distributed in the cytosol and accumulated at the plasma membrane, especially at regions of transient intercellular contacts. GFP-RacF1 also localized transiently to macropinosomes and phagocytic cups and was gradually released within <1 min after formation of the endocytic vesicle or the phagosome, respectively. On stimulation with cAMP, no enrichment of GFP-RacF1 was observed in leading fronts, from which it was found to be initially excluded. Cell lines were obtained using homologous recombination that expressed a truncated racF1 gene lacking sequences encoding the carboxyl-terminal region responsible for membrane targeting. These cells displayed normal phagocytosis, endocytosis, and exocytosis rates. Our results suggest that RacF1 associates with dynamic structures that are formed during pinocytosis and phagocytosis. Although RacF1 appears not to be essential, it might act in concert and/or share functions with other members of the Rho family in the regulation of a subset of cytoskeletal rearrangements that are required for these processes.

L10 ANSWER 23 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:65014 The Genuine Article (R) Number: 155MR. Multiple signal transduction pathways regulate TNF-induced actin reorganization in macrophages: Inhibition of **Cdc42**-mediated filopodium formation by TNF. Peppelenbosch M (Reprint); Boone E; Jones G E; vanDeventer S J H; Haegeman G; Fiers W; Grooten J; Ridley A J. UNIV AMSTERDAM, ACAD MED CTR, LAB EXPT INTERNAL MED, MEIBERGDREEF 9, G2-130, NL-1105 AZ AMSTERDAM,

NETHERLANDS (Reprint); LUDWIG INST CANC RES, LONDON W1P 8BT, ENGLAND; UNIV AMSTERDAM, ACAD MED CTR, LAB EXPT INTERNAL MED, NL-1105 AZ AMSTERDAM, NETHERLANDS; FLEMISH INST BIOTECHNOL, MOL BIOL LAB, GHENT, BELGIUM; UNIV LONDON KINGS COLL, RANDALL INST, LONDON WC2R 2LS, ENGLAND; UNIV COLL LONDON, DEPT BIOCHEM & MOL BIOL, LONDON, ENGLAND. JOURNAL OF IMMUNOLOGY (15 JAN 1999) Vol. 162, No. 2, pp. 837-845. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0022-1767. Pub. country: NETHERLANDS; ENGLAND; BELGIUM. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB TNF is known to regulate macrophage (M phi) migration, but the signaling pathways mediating this response have not been established. Here we report that stimulation of the 55-kDa TNF receptor (TNFR-1) induced an overall decrease in filamentous actin (F-actin), inhibited CSF-1- and **Cdc42**-dependent filopodium formation, and stimulated macropinocytosis. Using a panel of TNFR-1 mutants, the regions of the receptor required for each of these responses were mapped. The decrease in F-actin required both the death domain and the membrane proximal part of the receptor, whereas inhibition of filopodium formation and increased pinocytosis were only dependent upon a functional death domain. When the TNF-induced decrease in F-actin was inhibited using either receptor mutants or the compound D609, TNF-stimulated actin reorganization at the cell cortex became apparent. This activity was dependent upon the FAN-binding region of TNFR-1, We conclude that different domains of TNFR-1 mediate distinct changes in the M phi cytoskeleton, and that the ability of TNF to inhibit M phi chemotaxis may be due to decreased filopodium formation downstream of **Cdc42**.

L10 ANSWER 24 OF 41 MEDLINE

2000054270 Document Number: 20054270. PubMed ID: 10585277. Activated Rac1 selectively up-regulates the expression of integrin alpha6beta4 and induces cell adhesion and membrane ruffles of nonadherent colon cancer Colo201 cells. Mohri T; Adachi Y; Ikehara S; Hioki K; Tokunaga R; Taketani S. (Second Department of Surgery, Kansai Medical University, Moriguchi, 570-8506, Japan.) EXPERIMENTAL CELL RESEARCH, (1999 Dec 15) 253 (2) 533-40. Journal code: EPB; 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB Functions of small GTPases in integrin expression were investigated when the interaction of nonadherent human colon carcinoma 201 cells with the extracellular matrix (ECM) was examined. By transfection of the constitutively active form of a small GTPase Rac1, Rac V12, adhesion of cells to the ECM increased with concomitant cell spreading and formation of membrane ruffles. Activated **Cdc42** and **Cdc42** V12, but not wild-type Rac1, **Cdc42**, or RhoA, also induced the adhesion and spreading of Colo201 cells. This adhesion is integrin beta4 dependent since an **antibody** for integrin beta4 inhibited the RacV12-dependent cell adhesion and numbers of adhesive cells on laminin-coated plates exceeded those on collagen- and fibronectin-coated plates. By immunofluorescence, in addition to clustering of integrin molecules, expression of integrin alpha6beta4 on the cell surface of Rac V12- and **Cdc42** V12-expressing cells was selectively up-regulated without an increase in biosynthesis of alpha6beta4 integrin. Treatment of Rac V12-expressing cells with wortmannin or LY294002, specific inhibitors of phosphoinositide 3-OH kinase, decreased the up-regulated alpha6beta4 and cell adhesion. In light of this evidence, we propose that the regulation of integrin alpha6beta4 expression induced by Rac1 and **Cdc42** may play an important role in cell adhesion and tumorigenesis of colon carcinoma cells.

L10 ANSWER 25 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)

1999:961684 The Genuine Article (R) Number: 263QE. Melanoma chondroitin sulphate proteoglycan regulates cell spreading through **Cdc42**, Ack-1 and p130(cas). Eisenmann K M; McCarthy J B (Reprint); Simpson M A;

Keely P J; Guan J L; Tachibana K; Lim L; Manser E; Furcht L T; Iida J. UNIV MINNESOTA, DEPT LAB MED & PATHOL, MINNEAPOLIS, MN 55455 (Reprint); UNIV MINNESOTA, DEPT LAB MED & PATHOL, MINNEAPOLIS, MN 55455; UNIV MINNESOTA, INST BIOMED ENGN, MINNEAPOLIS, MN 55455; UNIV MINNESOTA, CTR CANC, MINNEAPOLIS, MN 55455; UNIV WISCONSIN, DEPT PHARMACOL, MADISON, WI 53706; CORNELL UNIV, DEPT MOL MED, ITHACA, NY 14853; HARVARD UNIV, SCH MED, DEPT CANC IMMUNOL & AIDS, BOSTON, MA 02115; NEUROL INST, LONDON WC1N 1PJ, ENGLAND; NATL UNIV SINGAPORE, INST MOL & CELL BIOL, SINGAPORE 119076, SINGAPORE. NATURE CELL BIOLOGY (DEC 1999) Vol. 1, No. 8, pp. 507-513. Publisher: MACMILLAN MAGAZINES LTD. PORTERS SOUTH, 4 CRINAN ST, LONDON N1 9XW, ENGLAND. ISSN: 1465-7392. Pub. country: USA; ENGLAND; SINGAPORE. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Melanoma chondroitin sulphate proteoglycan (MCSP) is a cell-surface antigen that has been implicated in the growth and invasion of melanoma tumours, Although this antigen is expressed early in melanoma progression, its biological function is unknown. MCSP can stimulate the integrin-alpha(4)beta(1)-mediated adhesion and spreading of melanoma cells. Here we show that stimulated MCSP recruits tyrosine-phosphorylated p130(cas), an adaptor protein important in tumour cell motility and invasion. MCSP stimulation also results in a pronounced activation and recruitment of the Rho-family GTPase **Cdc42**. MCSP-induced spreading of melanoma cells is dependent upon active **Cdc42**, a **Cdc42**-associated tyrosine kinase (Ack-1) and tyrosine phosphorylation of p130(cas). Furthermore, vectors inhibiting Ack-1 or **Cdc42** expression and/or function abrogate MCSP-induced tyrosine phosphorylation and recruitment of p130(cas). Our findings indicate that MCSP may modify tumour growth or invasion by a unique signal-transduction pathway that links **Cdc42** activation to downstream tyrosine phosphorylation and subsequent cytoskeletal reorganization.

L10 ANSWER 26 OF 41 CAPLUS COPYRIGHT 2002 ACS

1998:605003 Document No. 129:199597 Human PARG is a GTPase-activating protein which interacts with protein tyrosine phosphatase L1. Saras, Jan; Franzen, Petra; Aspenstrom, Pontus; Hellman, Ulf; Gonez, Leonel Jorge; Heldin, Carl-henrik (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 9837196 A1 19980827, 93 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US3323 19980219. PRIORITY: US 1997-805583 19970225.

AB The invention describes nucleic acids encoding the PARG protein, including fragments and biol. functional variants thereof. PARG is a novel 150-kDa protein, the 4 most C-terminal amino acid residues of which specifically interact with the fourth PDZ domain of PLPL1 phosphatase. The mol. contains a GTPase-activating protein (GAP) domain, a cysteine-rich putative Zn2+- and diacylglycerol-binding domain, and a region of sequence homol. to the product of Caenorhabditis elegans gene ZK669.1a. The GAP domain is active on Rho, Rac, and **Cdc42** in vitro but with a clear preference for Rho. A complex between PTPL1 and PARG may function as a powerful neg. regulator of Rho signaling, acting both on Rho itself and on tyrosine phosphorylated components in the Rho signal transduction pathway. Also included are polypeptides and fragments thereof encoded by PARG-encoding nucleic acids, and **antibodies** relating thereto.

L10 ANSWER 27 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)

1998:326307 The Genuine Article (R) Number: ZJ519. Two signaling mechanisms for activation of alpha(M)beta(2) avidity in polymorphonuclear neutrophils . Jones S L; Knaus U G; Bokoch G M; Brown E J (Reprint). WASHINGTON UNIV, SCH MED, DIV INFECT DIS, BOX 8051, 660 S EUCLID AVE, ST LOUIS, MO 63110 (Reprint); WASHINGTON UNIV, SCH MED, DIV INFECT DIS, ST LOUIS, MO 63110; SCRIPPS CLIN, DEPT IMMUNOL, LA JOLLA, CA 92037; SCRIPPS CLIN & RES INST, DEPT IMMUNOL, LA JOLLA, CA 92037; SCRIPPS CLIN & RES INST, DEPT CELL BIOL, LA JOLLA, CA 92037. JOURNAL OF BIOLOGICAL CHEMISTRY (24 APR 1998) Vol.

273, No. 17, pp. 10556-10566. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0021-9258. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Circulating polymorphonuclear neutrophils (PMN) are quiescent, nonadherent cells that rapidly activate at sites of inflammation, where they develop the capacity to perform a repertoire of functions that are essential for host defense. Induction of integrin-mediated adhesion, which requires an increase in integrin avidity, is critical for the development of these effector functions. Although a variety of stimuli can activate integrins in PMN, the signaling cascades involved are unclear. Phosphatidylinositol (PI) 3-kinase has been implicated in integrin activation in a variety of cells, including PMN. In this work, we have examined activation of the PMN integrin alpha(M) beta(2), assessing both adhesion and generation of the epitope recognized by the activation-specific **antibody** CBRM1/5. We have found that PI 3-kinase has a role in activation of alpha(M) beta(2) by immune complexes, but we have found no role for it in alpha(M) beta(2) activation by ligands for trimeric G protein-coupled receptors, including formylmethionylleucylphenylalanine (fMLP), interleukin-8, and C5a. Cytochalasin D inhibition suggests a role for the actin cytoskeleton in immune complex activation of alpha(M) beta(2), but cytochalasin has no effect on fMLP-induced activation. Similarly, immune complex activation of the Rac/**Cdc42**-dependent serine/threonine kinase Pak1 is blocked by PI 3-kinase inhibitors, but fMLP-induced activation is not. These results demonstrate that two signaling pathways exist in PMN for activation of alpha(M) beta(2)-One, induced by Fc gamma R ligation, is PI 3-kinase-dependent and requires the actin cytoskeleton. The second, initiated by G protein-linked receptors, is PI 3-kinase-independent and cytochalasin-insensitive. Pak1 may be in a final common pathway leading to activation of alpha(M) beta(2).

L10 ANSWER 28 OF 41 MEDLINE DUPLICATE 4
1998378568 Document Number: 98378568. PubMed ID: 9710640. Ras-GAP controls Rho-mediated cytoskeletal reorganization through its SH3 domain. Leblanc V; Tocque B; Delumeau I. (Rhone-Poulenc Rorer Central Research, Gene Medicine Department, Centre de Recherche de Vitry Alfortville, 94403 Vitry sur Seine, France.. veronique.leblanc@exonhit.com) . MOLECULAR AND CELLULAR BIOLOGY, (1998 Sep) 18 (9) 5567-78. Journal code: NGY; 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB Proteins of the Ras superfamily, Ras, Rac, Rho, and **Cdc42**, control the remodelling of the cortical actin cytoskeleton following growth factor stimulation. A major regulator of Ras, Ras-GAP, contains several structural motifs, including an SH3 domain and two SH2 domains, and there is evidence that they harbor a signalling function. We have previously described a **monoclonal antibody** to the SH3 domain of Ras-GAP which blocks Ras signalling in Xenopus oocytes. We now show that microinjection of this **antibody** into Swiss 3T3 cells prevents the formation of actin stress fibers stimulated by growth factors or activated Ras, but not membrane ruffling. This inhibition is bypassed by coinjection of activated Rho, suggesting that the Ras-GAP SH3 domain is necessary for endogenous Rho activation. In agreement, the **antibody** blocks lysophosphatidic acid-induced neurite retraction in differentiated PC12 cells. Furthermore, we demonstrate that microinjection of full-length Ras-GAP triggers stress fiber polymerization in fibroblasts in an SH3-dependent manner, strongly suggesting an effector function besides its role as a Ras downregulator. These results support the idea that Ras-GAP connects the Ras and Rho pathways and, therefore, regulates the actin cytoskeleton through a mechanism which probably does not involve p190 Rho-GAP.

L10 ANSWER 29 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:498869 The Genuine Article (R) Number: ZW151. Rac regulates

integrin-mediated spreading and increased adhesion of T lymphocytes. DSouzaSchorey C; Boettner B; VanAelst L (Reprint). COLD SPRING HARBOR LAB, 1 BUNGTOWN RD, COLD SPRING HARBOR, NY 11724 (Reprint); COLD SPRING HARBOR LAB, COLD SPRING HARBOR, NY 11724; WASHINGTON UNIV, SCH MED, DEPT CELL BIOL, ST LOUIS, MO 63110. MOLECULAR AND CELLULAR BIOLOGY (JUL 1998) Vol. 18, No. 7, pp. 3936-3946. Publisher: AMER SOC MICROBIOLOGY. 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171. ISSN: 0270-7306. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Leukocyte adhesion to the extracellular matrix (ECM) is tightly controlled and is vital for the immune response. Circulating lymphocytes leave the bloodstream and adhere to ECM components at sites of inflammation and lymphoid tissues. Mechanisms for regulating T-lymphocyte-ECM adhesion include (i) an alteration in the affinity of cell surface integrin receptors for their extracellular ligands and (ii) an alteration of events following postreceptor occupancy (e.g., cell spreading). Whereas H-Ras and R-Ras were previously shown to affect T-cell adhesion by altering the affinity state df the integrin receptors, no signaling molecule has been identified for the second mechanism. In this study, we demonstrated that expression of an activated mutant of Rac triggered dramatic spreading of T cells and their increased adhesion on immobilized fibronectin in an integrin-dependent manner. This effect was not mimicked by expression of activated mutant forms of Rho, **Cdc42**, H-Ras, oi ARF6, indicating the unique role of pac in this event. The Rac-induced spreading was accompanied by specific cytoskeletal rearrangements; Also, a clustering of integrins at sites of cell adhesion and at the peripheral edges of spread cells was observed. We demonstrate that expression of RacV12 did not alter the level of expression of cell surface integrins or the affinity state of the integrin receptors. Moreover, our results indicate that Rac plays a role in the regulation of T-cell adhesion by a mechanism involving cell spreading, rather than by altering the level of expression or the affinity of the integrin receptors. Furthermore, we show that the Rac-mediated signaling pathway leading to spreading of T lymphocytes did not require activation of c-Jun kinase, serum response factor, or pp70(S6) (kinase) but appeared to involve a phospholipid kinase.

L10 ANSWER 30 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)

1998:893658 The Genuine Article (R) Number: 139ZU. The IQGAP-related protein DGAP1 interacts with Rac and is involved in the modulation of the F-actin cytoskeleton and control of cell motility. Faix J (Reprint); Clougherty C; Konzok A; Mintert U; Murphy J; Albrecht R; Muhlbauer B; Kuhlmann J. MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED, GERMANY (Reprint); MAX PLANCK INST MOL PHYSIOL, D-44026 DORTMUND, GERMANY. JOURNAL OF CELL SCIENCE (OCT 1998) Vol. 111, Part 20, pp. 3059-3071. Publisher: COMPANY OF BIOLOGISTS LTD. BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND. ISSN: 0021-9533. Pub. country: GERMANY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB DGAP1 of Dictyostelium discoideum is a cell cortex associated 95 kDa protein that shows homology to both RasGTPase-activating proteins (RasGAPs) and RasGAP-related proteins. When tested for RasGAP activity, recombinant DGAP1 protein did not promote the GTPase activity of human H-Ras or of Dictyostelium RasG in vitro. Instead, DGAP1 bound to Dictyostelium Rac1A and human Rad, but not to human **Cdc42**. DGAP1 preferentially interacted with the activated GTP-bound forms of Rad and Rac1A, but did not affect the GTPase activities. Since Rho-type GTPases are implicated in the formation of specific F-actin structures and in the control of cell morphology, the microfilament system of mutants that either lack or overexpress DGAP1 has been analysed. DGAP1-null mutants showed elevated levels of F-actin that was organised in large leading edges, membrane ruffles or numerous large filopods. Expression of actin fused to green fluorescent protein (GFP) was used to monitor the actin

dynamics in these cells, and revealed that the F-actin cytoskeleton of DGAP1-null cells was rapidly re-arranged to form ruffles and filopods, Conversely, in DGAP1-overexpressing cells, the formation of cellular projections containing F-actin was largely suppressed. Measurement of cell migration demonstrated that DGAP1 expression is inversely correlated with the speed of cell motility.

L10 ANSWER 31 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:60570 The Genuine Article (R) Number: 154QQ. Visualization and molecular analysis of actin assembly in living cells. Schafer D A (Reprint); Welch M D; Machesky L M; Bridgman P C; Meyer S M; Cooper J A. WASHINGTON UNIV, SCH MED, DEPT CELL BIOL & PHYSIOL, 660 S EUCLID AVE, ST LOUIS, MO 63110 (Reprint); WASHINGTON UNIV, SCH MED, DEPT ANAT & NEUROBIOL, ST LOUIS, MO 63110; UNIV CALIF BERKELEY, BERKELEY, CA 94702; UNIV BIRMINGHAM, SCH BIOCHEM, BIRMINGHAM B15 2TT, W MIDLANDS, ENGLAND. JOURNAL OF CELL BIOLOGY (28 DEC 1998) Vol. 143, No. 7, pp. 1919-1930. Publisher: ROCKEFELLER UNIV PRESS. 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021. ISSN: 0021-9525. Pub. country: USA; ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Actin filament assembly is critical for eukaryotic cell motility. Arp2/3 complex and capping protein (CP) regulate actin assembly in vitro. To under stand how these proteins regulate the dynamics of actin filament assembly in a motile cell, we visualized their distribution in living fibroblasts using green flourescent protein (GFP) tagging. Both proteins were concentrated in motile regions at the cell periphery and at dynamic spots within the lamella. Actin assembly was required for the motility and dynamics of spots and for motility at the cell periphery. In permeabilized cells, rhodamine-actin assembled at the cell periphery and at spots, indicating that actin filament barbed ends were present at these locations. Inhibition of the Rho family GTPase rad, and to a lesser extent **cdc42** and RhoA, blocked motility at the cell periphery and the formation of spots. Increased expression of phosphatidylinositol 5-kinase promoted the movement of spots. Increased expression of LIM-kinase-1, which likely inactivates cofilin, decreased the frequency of moving spots and led to the formation of aggregates of GFP-CP. We conclude that spots, which appear as small projections on the surface by whole mount electron microscopy, represent sites of actin assembly where local and transient changes in the cortical actin cytoskeleton take place.

L10 ANSWER 32 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:463581 The Genuine Article (R) Number: ZT687. B-50/GAP-43-induced formation of filopodia depends on Rho-GTPase. Aarts L H J (Reprint); Schrama L H; Hage W J; Bos J L; Gispen W H; Schotman P. UNIV UTRECHT, DEPT PHYSIOL CHEM, NL-3584 CG UTRECHT, NETHERLANDS (Reprint); UNIV UTRECHT, RUDOLF MAGNUS INST NEUROSCI, NL-3584 CG UTRECHT, NETHERLANDS; UNIV UTRECHT, RUDOLF MAGNUS INST NEUROSCI, DEPT MED PHARMACOL, NL-3584 CG UTRECHT, NETHERLANDS; NETHERLANDS INST DEV BIOL, HUBRECHT LAB, NL-3584 CG UTRECHT, NETHERLANDS. MOLECULAR BIOLOGY OF THE CELL (JUN 1998) Vol. 9, No. 6, pp. 1279-1292. Publisher: AMER SOC CELL BIOLOGY. PUBL OFFICE, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 1059-1524. Pub. country: NETHERLANDS. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Ln the present study we show that expression of the neural PKC-substrate B-50 (growth-associated protein [GAP-43]) in Rat-1 fibroblasts induced the formation of filopodial extensions during spreading. This morphological change was accompanied by an enhanced formation of peripheral actin filaments and by accumulation of vinculin immunoreactivity in filopodial focal adhesions, colocalizing with B-50. In time lapse experiments, the B50-induced filopodial extensions were shown to stay in close contact with the substratum and appeared remarkably stable, resulting in a delayed lamellar spreading of the fibroblasts. The morphogenetic effects of the B-50 protein were entirely dependent on the integrity of the two N-terminal cysteines involved in membrane association

(C3C4), but were not significantly affected by mutations of the PKC-phosphorylation site (S41) or deletion of the C terminus (177-226). Cotransfection of B-50 with dominant negative **Cdc42** or Rac did not prevent B-50-induced formation of filopodial cells, whereas this process could be completely blocked by cotransfection with dominant negative Rho or Clostridium botulinum C3-transferase. Conversely, constitutively active Rho induced a similar filopodial phenotype as B-50. We therefore propose that the induction of surface extensions by B-50 in spreading Rat-1 fibroblasts depends on Rho-guanosine triphosphatase function.

L10 ANSWER 33 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)

1998:376181 The Genuine Article (R) Number: ZM927. Vav is a regulator of cytoskeletal reorganization mediated by the T-cell receptor. Fischer K D (Reprint); Kong Y Y; Nishina H; Tedford K; Marengere L E M; Kozieradzki I; Sasaki T; Starr M; Chan G; Gardener S; Nghiem M P; Bouchard D; Barbacid M; Bernstein A; Penninger J M. UNIV WURZBURG, INST MED STRAHLENKUNDE & ZELLFORSCH, VERSBACHER STR 5, D-97078 WURZBURG, GERMANY (Reprint); UNIV TORONTO, ONTARIO CANC INST, AMGEN INST, DEPT MED BIOPHYS, TORONTO, ON M5G 2C1, CANADA; UNIV TORONTO, ONTARIO CANC INST, AMGEN INST, DEPT IMMUNOL, TORONTO, ON M5G 2C1, CANADA; HOSP SICK CHILDREN, DEPT PATHOL, TORONTO, ON M5G 1X8, CANADA; MT SINAI HOSP, SAMUEL LUNENFELD RES INST, MT SINAI HOSP, TORONTO, ON M5G 1X5, CANADA; MT SINAI HOSP, DEPT MED GENET, TORONTO, ON M5G 1X5, CANADA; BRISTOL MYERS SQUIBB PHARMACEUT RES INST, DEPT MOL BIOL, PRINCETON, NJ 08543. CURRENT BIOLOGY (7 MAY 1998) Vol. 8, No. 10, pp. 554-562. Publisher: CURRENT BIOLOGY LTD. 34-42 CLEVELAND STREET, LONDON W1P 6LB, ENGLAND. ISSN: 0960-9822. Pub. country: GERMANY; CANADA; USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Vav is a guanine-nucleotide exchange factor for the Rho-like small GTPases RhoA, Rac1 and **Cdc42**, which regulate cytoskeletal reorganization and activation of stress-activated protein kinases (SAPK/JNKs). Vav is expressed in hematopoietic cells and is phosphorylated in T and B cells following activation of various growth factor or antigen receptors. Vav interacts with several signaling molecules in T cells, but the functional relevance of these interactions is established only for Slp76: they cooperate to induce activity of the transcription factor NF-AT and interleukin-2 expression. We have investigated the role of Vav in T cells by generating vav(-/-)mice.

Results: Mice deficient for vav were viable and healthy, but had impaired T-cell development. In vav(-/-) T cells, in response to activation of the T-cell receptor (TCR), cell cycle progression, induction of NF-ATc1 activity, downregulation of the cell-cycle inhibitor p27(Kip1) interleukin-2 production, actin polymerization and the clustering of TCRs into patches and caps - a cytoskeletal reorganization process - were defective. TCR-mediated activation of mitogen-activated protein kinase and SAPK/JNK was unaffected. Ca2+ mobilization was impaired in vav(-/-) thymocytes and T cells. In wild-type cells, Vav constitutively associated with the cytoskeletal membrane anchors talin and vinculin. In the absence of Vav, phosphorylation of Slp76, Slp76-talin interactions, and recruitment of the actin cytoskeleton to the CD3 xi chain of the TCR co-receptor were impaired.

Conclusions: Vav is a crucial regulator of TCR-mediated Ca2+ flux, cytoskeletal reorganization and TCR clustering, and these are required for T-cell maturation, interleukin-e production and cell cycle progression. (C) Current Biology Ltd ISSN 0960-9822.

L10 ANSWER 34 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)

1998:834130 The Genuine Article (R) Number: 132PM. Binding of myosin essential light chain to the cytoskeleton-associated protein IQGAP1. Weissbach L (Reprint); Bernards A; Herion D W. HARVARD UNIV, SCH MED, MASSACHUSETTS GEN HOSP, ORTHOPAED RES LABS, BOSTON, MA 02114 (Reprint); HARVARD UNIV, SCH MED, MASSACHUSETTS GEN HOSP, CTR CANC, BOSTON, MA 02114.

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (9 OCT 1998) Vol. 251, No. 1, pp. 269-276. Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0006-291X. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The 190 kD human IQGAP1 protein, by virtue of its N-terminal calponin-homology domain, is found associated with the actin cytoskeleton, and is capable of cross-linking actin filaments. IQGAP1 complexes with several proteins, including the Rho family GTPases **Cdc42** and Rac, as well as calmodulin. It was previously noted that one of the IQ motifs of IQGAP1 displays significant similarity to a myosin heavy chain IQ motif responsible for binding the calmodulin-related myosin essential light chain (ELC). Employing the yeast two-hybrid methodology as well as in vitro binding experiments, we present evidence that a truncated version of IQGAP1 can interact with the myosin ELC. This interaction may have significant consequences for various cellular processes that involve actomyosin contractility, and suggests that the biological targets of the ELC may not be restricted to the myosin heavy chain. (C) 1998 Academic Press.

L10 ANSWER 35 OF 41 CAPLUS COPYRIGHT 2002 ACS
1997:425363 Document No. 127:32828 Therapeutic and diagnostic vaccine for the treatment of microbial infections. Pascual, David; Bond, Clifford; Burritt, James; Burgess, Don; Glee, Pati; Jutila, John; Jutila, Mark; Bargatze, Robert; Mcfeters, Gordon; Pyle, Barry; Cutler, Jim E.; Han, Yongmoon (Research and Development Institute, Inc., USA; Pascual, David; Bond, Clifford; Burritt, James; Burgess, Don; Glee, Pati; Jutila, John; Jutila, Mark; Bargatze, Robert; et al.). PCT Int. Appl. WO 9718790 A2 19970529, 98 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US18796 19961121. PRIORITY: US 1995-7477 19951122.

AB Therapeutic peptides, vaccines and diagnostic agents are disclosed for the treatment of pathogenic infections. The agents are capable of binding to mol. address on host cell (e.g. leukocyte, endothelial or epithelial cells, nerve cells), triggering one or more signal transduction pathways and enabling selective pathogen or toxin to traffic through host tissue. The agents are microbial attachment mols. such as adhesive protein, glycoprotein, lectin, carbohydrate, glycolipid.

L10 ANSWER 36 OF 41 CAPLUS COPYRIGHT 2002 ACS
1997:440191 Document No. 127:48829 A cDNA for the .beta.APP-C100 receptor for the C-terminal fragment of .beta.-amyloid precursor and the treatment and prophylaxis of Alzheimer's disease. Manly, Susan P.; Kozlowski, Michael R.; Neve, Rachael L. (Bristol-Myers Squibb Company, USA; Mclean Hospital Corporation). PCT Int. Appl. WO 9718230 A1 19970522, 110 pp. DESIGNATED STATES: W: CA, JP, MX; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US18572 19961115. PRIORITY: US 1995-559397 19951115.

AB A cDNA for the receptor for the C-terminal 100 amino acid fragment of the amyloid precursor protein is cloned and expressed and systems are developed to screen for inhibitors of the interaction that may be useful in the treatment or prophylaxis of Alzheimer's disease. Such engineered cells may be used to evaluate and screen drugs and analogs of .beta.-APP involved in Alzheimer's Disease. A cDNA for the receptor was cloned by screening a rat fetal brain cDNA expression library in .lambda.gt11 by screening with labeled peptide. In vitro translated receptor was shown to bind **Cdc42** and Rac1 when they had been activated by binding GTP, but not by binding GDP and appears to be a kinase. The mRNA was present

in a no. of neural tissues with a tissue distribution matching that of the amyloid precursor mRNA.

L10 ANSWER 37 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
97:759667 The Genuine Article (R) Number: YA401. Selective control of membrane ruffling and actin plaque assembly by the Rho GTPases Rac1 and **CDC42** in Fc epsilon RI-activated rat basophilic leukemia (RBL-2H3) cells. Guillemot J C; Montcourrier P; Vivier E; Davoust J; Chavrier P (Reprint). CNRS, INSERM, CTR IMMUNOL, CASE 906, F-13288 MARSEILLE 9, FRANCE (Reprint); CNRS, INSERM, CTR IMMUNOL, F-13288 MARSEILLE 9, FRANCE; UNIV MONTPELLIER 2, CNRS, UMR 5539, F-34095 MONTPELLIER 5, FRANCE. JOURNAL OF CELL SCIENCE (SEP 1997) Vol. 110, Part 18, pp. 2215-2225. Publisher: COMPANY OF BIOLOGISTS LTD. BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE, CAMBS, ENGLAND CB4 4DL. ISSN: 0021-9533. Pub. country: FRANCE. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Engagement of the high affinity IgE receptor (Fc epsilon RI) in mast cells elicits a series of intracellular signalling events including cytoskeletal reorganization and granule exocytosis, To analyze the coupling of receptor activation to specific cytoskeletal responses, we expressed dominant negative mutant forms of the Rho GTPases **CDC42** and Rad in rat RBL-2H3 tumor mast cells, We show here that dominant inhibition of **CDC42** function decreases cell adhesion, interferes with Fc epsilon RI-induced actin plaque assembly and reduced the recruitment of vinculin at the cell-substratum interface, while the inhibitory Rad mutant abolishes Fc epsilon RI-mediated membrane ruffling. The expression of trans-dominant inhibitory for-ms of either **CDC42** or Rad significantly inhibited antigen-induced degranulation, Altogether, our results demonstrate that **CDC42** and Rad control distinct pathways downstream of Fc epsilon RI engagement leading either to the induction of actin plaques, or to the production of membrane ruffles, These two pathways are critically involved during the degranulation response induced by Fc epsilon RI aggregation.

L10 ANSWER 38 OF 41 MEDLINE DUPLICATE 5
97294604 Document Number: 97294604. PubMed ID: 9150360. Actin cytoskeleton polymerization in Dbl-transformed NIH3T3 fibroblasts is dependent on cell adhesion to specific extracellular matrix proteins. Defilippi P; Olivo C; Tarone G; Mancini P; Torrisi M R; Eva A. (Dipartimento di Genetica, Biologia e Chimica Medica, Universita di Torino, Italy.) ONCOGENE, (1997 Apr 24) 14 (16) 1933-43. Journal code: ONC; 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The Dbl oncogene is the putative exchange factor for two small GTP-binding proteins, RhoA and **CDC42** which are involved in the polymerization of actin to produce stress fibers and filopodia, respectively. We report here that Dbl oncogene-transformed NIH3T3 cells show actin stress fibers only when cells are plated on fibronectin. Plating of cells on collagen I and IV as well as on poly-D-lysine and gelatin induces polymerization of actin to form filopodia, lamellipodia and membrane ruffles but not stress fibers. The putative collagen receptors, alpha1/beta1 and alpha2/beta1 integrins are expressed at reduced level in Dbl-transformed cells compared to untransformed NIH3T3 fibroblasts. Nevertheless, adhesion to collagens is not altered. Inhibitory **monoclonal antibody** to mouse integrin beta1 subunit blocked adhesion of both Dbl-transformed and untransformed NIH3T3 cells, demonstrating that adhesion to collagen I and IV is mediated by the beta1 family of integrins. Dbl product rapidly induces the depolymerization of actin stress fibers, rounding up of the cells, and formation of filopodia and lamellipodia when microinjected in NIH3T3 cells plated on gelatin. Thus, Dbl may exert its effect on actin cytoskeleton organization in response to extracellular proteins by altering integrin-mediated signalling pathways.

L10 ANSWER 39 OF 41 SCISEARCH COPYRIGHT 2002 ISI (R)
96:779497 The Genuine Article (R) Number: VP233. MAMMALIAN **CDC42** IS A BREFELDIN A-SENSITIVE COMPONENT OF THE GOLGI-APPARATUS. ERICKSON J W (Reprint); ZHANG C J; KAHN R A; EVANS T; CERIONE R A. CORNELL UNIV, COLL VET MED, DEPT PHARMACOL, ITHACA, NY, 14853 (Reprint); NCI, BIOL CHEM LAB, DEV THERAPEUT PROGRAM, DIV CANC TREATMENT, NIH, BETHESDA, MD, 20892; ONYX PHARMACEUT, RICHMOND, CA, 94806. JOURNAL OF BIOLOGICAL CHEMISTRY (25 OCT 1996) Vol. 271, No. 43, pp. 26850-26854. ISSN: 0021-9258. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In this study, we have used immunocytochemical and fractionation approaches to provide a description oft he localization of the mammalian **Cdc42** protein (designated Cdc42Hs) in vivo. A specific anti-peptide **antibody** was generated against the C-terminal region of Cdc42Hs. Using affinity-purified preparations of this **antibody** in indirect immunofluorescence experiments, Cdc42Hs was found to be localized to the Golgi apparatus, Similar to the well-characterized non-clathrin coat proteins ADP-ribosylation factor (ARF) and beta-COP, the perinuclear clustering of Cdc42Hs is rapidly dispersed upon exposure of the cells to the drug brefeldin A, suggesting that it too may play a role in the processes of intracellular lipid and protein transport, Employing cell lines possessing inducible forms of ARF, we demonstrate here a tight coupling of the nucleotide bound state of ARF and the subcellular localization of Cdc42Hs. Specifically, the expression of wild-type ARF had no effect on the brefeldin A sensitivity of Cdc42Hs while, as is the case for ARF and P-COP, expression of a GTPase-deficient form of ARF (ArF(Q71L)) renders these Golgi-localized proteins resistant to brefeldin A treatment (Teal et al., 1994; Zhang et al,, 1994). Moreover, the induced expression of a mutant form of ARF with a low affinity for nucleotide resulted in constitutive redistribution of Cdc42Hs in the absence of brefeldin A treatment. These results suggest that Cdc42Hs may play a role in cell morphogenesis by acting on targets in the Golgi that direct polarized growth at the plasma membrane.

L10 ANSWER 40 OF 41 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
95354786 EMBASE Document No.: 1995354786. **Cdc42** and PAK-mediated signaling leads to Jun kinase and p38 mitogen- activated protein kinase activation. Bagrodia S.; Derijard B.; Davis R.J.; Cerione R.A.. Department of Pharmacology, Cornell University,Ithaca, NY 14853-6401, United States. Journal of Biological Chemistry 270/47 (27995-27998) 1995. ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB The PAK family of protein kinases has been suggested as a potential target of the **Cdc42** and Rac GTPases based on studies in vitro. We show that PAK-3 is activated by **Cdc42** in vivo. Both, activated (GTPase-defective) **Cdc42** and a constitutively active PAK-3 mutant stimulated the activity of Jun kinase 1 (JNK1) in transfected cells. Activated **Cdc42** also stimulated the activity of the related p38 mitogen-activated protein kinase but was a less effective activator of ERK2. The effect of **Cdc42** on JNK activity was similar to that of the potent inflammatory cytokine interleukin-1 (IL-1). The observation that a dominant-negative **Cdc42** mutant inhibited IL-1 activation of JNK1 indicates a role for **Cdc42** in IL-1 signaling. These results suggest that **Cdc42** and PAK may mediate the effects of cytokines on transcriptional regulation.

L10 ANSWER 41 OF 41 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
94272116 EMBASE Document No.: 1994272116. Distinct morphogenetic functions of similar small GTPases: Drosophila Drac1 is involved in axonal outgrowth and myoblast fusion. Luo L.; Liao Y.J.; Lily Yeh Jan; Yuh Nung Jan. Department of Physiology, Howard Hughes Medical Institute, University of California,San Francisco, CA 94143, United States. Genes and Development

8/15 (1787-1802) 1994.
ISSN: 0890-9369. CODEN: GEDEEP. Pub. Country: United States. Language: English. Summary Language: English.

AB The small GTPases of the Rac/Rho/**Cdc42** subfamily are implicated in actin cytoskeleton-membrane interaction in mammalian cells and budding yeast. The in vivo functions of these GTPases in multicellular organisms are not known. We have cloned Drosophila homologs of rac and **CDC42**, Drac1, and Dcdc42. They share 70% amino acid sequence identity with each other, and both are highly expressed in the nervous system and mesoderm during neuronal and muscle differentiation, respectively. We expressed putative constitutively active and dominant-negative Drac1 proteins in these tissues. When expressed in neurons, Drac1 mutant proteins cause axon outgrowth defects in peripheral neurons without affecting dendrites. When expressed in muscle precursors, they cause complete failure of, or abnormality in, myoblast fusion. Expressions of analogous mutant Dcdc42 proteins cause qualitatively distinct morphological defects, suggesting that similar GTPases in the same subfamily have unique roles in morphogenesis.

=> s l1 and thyroid hormone receptor
L11 631 L1 AND THYROID HORMONE RECEPTOR

=> s l11 and monoclonal
L12 131 L11 AND MONOCLONAL

=> s l12 and polyclonal
L13 14 L12 AND POLYCLONAL

=> dup remove l13
PROCESSING COMPLETED FOR L13
L14 6 DUP REMOVE L13 (8 DUPLICATES REMOVED)

=> d l14 1-6 cbib abs

L14 ANSWER 1 OF 6 MEDLINE DUPLICATE 1
97271687 Document Number: 97271687. PubMed ID: 9126603. One-step immunoaffinity purification of recombinant human retinoic acid receptor gamma. Repa J J; Berg J A; Kaiser M E; Hanson K K; Strugnell S A; Clagett-Dame M. (Interdepartmental Graduate Program in Nutritional Sciences, University of Wisconsin-Madison 53706, USA.) PROTEIN EXPRESSION AND PURIFICATION, (1997 Apr) 9 (3) 319-30. Journal code: BJV; 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Retinoic acid receptors (RAR) are members of the steroid/**thyroid hormone receptor** superfamily and serve as ligand-activated transcription factors. In order to facilitate studies of receptor protein, we have generated a **monoclonal antibody** to the human RAR gamma, and have developed a procedure to purify the full-length receptor expressed in insect cells. The **monoclonal antibody** (A10) was developed using as antigen a carboxy-terminal fragment of the human RAR gamma expressed as a bacterial fusion protein. The A10 **monoclonal antibody** binds to both native and denatured forms of the human RAR gamma. This **antibody** was immobilized on a resin and used to purify full-length, baculovirus-expressed human RAR gamma to near homogeneity. The immunoaffinity-purified receptor is > 90-95% pure as revealed by silver-stained gels. The identity of the single protein band as RAR gamma was verified by immunoblotting using a **polyclonal antibody** to an epitope distinct from that recognized by the A10 **antibody**. The pure human RAR gamma is functional with respect to both ligand and DNA binding. Scatchard analysis of 3H-labeled all-trans retinoic acid binding to purified human RAR gamma revealed a single, high-affinity binding site with a Kd of approximately 2 nM. Binding of the

pure RAR gamma to a DR5-type retinoic acid response element was also studied. Response element binding by RAR gamma required the presence of the retinoid X receptor, but did not require the presence of additional proteins. Human RAR gamma protein purified in this fashion will be useful in future structural and functional studies.

L14 ANSWER 2 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
95259440 EMBASE Document No.: 1995259440. Structure of the carboxy-terminal region of thyroid hormone nuclear receptors and its possible role in hormone-dependent intermolecular interactions. Bhat M.K.; McPhie P.; Ting Y.-T.; Zhu X.-G.; Cheng S.-Y.. Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, 37 Convent Drive,Bethesda, MD 20892-4255, United States. Biochemistry 34/33 (10591-10599) 1995.
ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.
AB The thyroid hormone nuclear receptors (TRs) are ligand-dependent transcription factors. To understand the molecular basis of ligand-dependent transactivation, we studied the structure of their carboxy-terminal activation domain. We analyzed the structures of the peptides derived from the C-terminal sequences of human TR subtypes .beta.1 (h-TR.beta.1) and .alpha.1 (h- TR.alpha.1) and a human TR mutant, PV, by circular dichroism (CD). Mutant PV has a C-terminal frameshift mutation and does not bind to the thyroid hormone, 3,3',5-triiodo-L-thyronine (T3). Analyses of the secondary structures of the peptides by CD indicate that five amino acids, EVFED, are part of an amphipathic .alpha.-helix which is required to maintain the structural integrity of the hormone binding domain. A **monoclonal antibody**, C4 (mAb C4), which recognizes both h-TR.beta.1 and h-TR.alpha.1 was developed. Using a series of truncated mutants and synthetic peptides, we mapped the epitope of mAb C4 to the conserved C-terminal amino acids, EVFED. Analysis of the binding data indicates that binding of T3 to either h-TR.beta.1 or h-TR.alpha.1 was competitively inhibited by mAb C4. Deletion of C-terminal amino acids including EVFED led to a total loss of T3 binding activity. Thus, part of the T3 binding site is located in this five amino acid segment. T3 may transduce its hormonal signal to the transcriptional machinery via interaction with EVFED at the C- terminus of TRs.

L14 ANSWER 3 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
95279592 EMBASE Document No.: 1995279592. Biological and immunochemical characterization of recombinant human thyrotrophin. Canonne C.; Papandreou M.-J.; Medri G.; Verrier B.; Ronin C.. Laboratoire d'Immunochimie, Hormones Glycoproteiques, Faculte de Medecine Nord,13916 Marseille Cedex 20, France. Glycobiology 5/5 (473-481) 1995.
ISSN: 0959-6658. CODEN: GLYCE3. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB Recombinant human thyroid-stimulating hormone (recTSH) has recently been engineered to detect metastatic lesions in patients operated on for thyroid cancer. In this report, we have compared the microheterogeneity, carbohydrate (CHO) content, mitogenic potency and immunoreactivity of the biotechnology product to those of human TSH of pituitary origin (pitTSH). Compositional analysis revealed that recombinant (rec) TSH produced in Chinese hamster ovary cells was overglycosylated compared with the native hormone (21 and 14%, respectively) with a higher amount of sialic acid and lack of N-acetylgalactosamine. Electrofocusing followed by immunoblotting resolved recTSH into six glycoforms with pIs ranging from 6.0 to 8.6, which were converted to a major species of pI 8.9 by sialidase treatment. pitTSH contained five main isoforms of pI 6.5-8.2 distinct from those of recTSH and partially resistant to sialidase. Binding activity of both human TSHs to porcine thyroid membrane receptors was found to be similar, but recTSH appeared to be 20% active compared to pitTSH in eliciting cAMP production and cell growth in rat FRTL-5 cells. Immunoreactivity of the

recombinant hormone was investigated using **polyclonal** and **monoclonal antibodies** raised against the native hormone or synthetic peptide sequences of its subunits. While rec- and pitTSH were recognized to a similar extent by anti-protein **antibodies**, they exhibited a different binding pattern to antipeptide **antibodies**. Serial dilution of anti-.alpha. 1-25, anti-.alpha. 26-51, anti-.beta. 96-112 antisera bound recTSH to a greater extent than pitTSH, while anti-.beta. 31-51 and anti-.beta. 53-76 displayed similar recognition toward both preparations. Inhibition assays showed that the .alpha. 1-25 and anti-.alpha. 26-51 regions contained at least two antigenic determinants which are present in recTSD but absent in the pituitary hormone. It is therefore concluded that recTSH differs from pitTSH with respect to several conformational features at the polypeptide surface, which are likely to be responsible for altered intrinsic bioactivity and may be potentially antigenic in patients repeatedly injected with the drug.

L14 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2002 ACS

1992:1748 Document No. 116:1748 Human thyroid-stimulating hormone receptor cDNA cloning and use in **monoclonal antibody** preparation. Milgrom, Edwin; Misrahi, Micheline; Loosfelt, Hugues; Atger, Michel (Institut National de la Sante et de la Recherche Medicale (INSERM), Fr.). PCT Int. Appl. WO 9110735 A2 19910725, 49 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (French). CODEN: PIXXD2. APPLICATION: WO 1991-FR25 19910115. PRIORITY: FR 1990-397 19900115.

AB A cDNA encoding the receptor for human TSH is cloned and expressed. The protein is used for the prepn. of **monoclonal** and **polyclonal antibodies** for use in the treatment of thyroid disorders (no data). The cDNA was cloned from a human thyroid cDNA bank in .lambda.gt10 by screening with porcine luteotropin/human chorionic gonadotropin receptor cDNA. Candidate clones were sequenced and confirmed by induction of thyroid hormone binding in COS cells. Transcription of the gene was limited to the thyroid gland. **Monoclonal antibodies** were prepd. against peptides from the hormone manufd. as fusion proteins with .beta.-galactosidase or with human ubiquitin in recombinant Escherichia coli.

L14 ANSWER 5 OF 6 MEDLINE DUPLICATE 2

89314401 Document Number: 89314401. PubMed ID: 2568604.

Immunocytochemical localization of thyroid hormone nuclear receptors in cultured hypothalamic dopaminergic neurons. Puymirat J; Luo M; Dussault J H. (Laboratoire d'Ontogenese et de genetique moleculaire, CHU Laval, Quebec, Canada.) NEUROSCIENCE, (1989) 30 (2) 443-9. Journal code: NZR; 7605074. ISSN: 0306-4522. Pub. country: ENGLAND: United Kingdom. Language: English.

AB By means of a **monoclonal antibody** against the rat liver L-triiodothyronine nuclear receptor and a **polyclonal** anti-tyrosine hydroxylase serum, it has been possible to demonstrate thyroid hormone nuclear receptors in immunoreactive tyrosine hydroxylase cell nuclei in fetal rat hypothalamic cultures. After 8 days in vitro, the ratio of tyrosine hydroxylase cells that were immunoreactive for the **thyroid hormone receptor** to those not stained for this receptor (64% to 36% respectively) remains unchanged despite an increase in the number of tyrosine hydroxylase-positive cells with time (from day 8 to day 21) in culture. The presence of thyroid hormone nuclear receptor in dopaminergic neurons is correlated with a morphological effect of L-triiodothyronine in this neuronal population. Our results demonstrate, for the first time, the presence of triiodothyronine nuclear receptors in fetal rat dopaminergic neurons and the existence of a cellular heterogeneity in the distribution of the **thyroid hormone receptor**. The presence of these receptors in fetal hypothalamic dopaminergic neurons suggests that some effects of

L-triiodothyronine on the maturation of DA neurons may result from a direct effect of this hormone through an interaction with its specific nuclear receptors.

L14 ANSWER 6 OF 6 MEDLINE DUPLICATE 3
89209214 Document Number: 89209214. PubMed ID: 2539925.
Immunocytochemical localization of nuclear 3,5,3'-triiodothyronine (L-T3) receptors in astrocyte cultures. Luo M; Puymirat J; Dussault J H. (Unite de Recherche en Ontogenese et Genetique Moleculaire, Le Centre Hospitalier de l'Universite Laval, Ste-Foy, Que., Canada.) BRAIN RESEARCH. DEVELOPMENTAL BRAIN RESEARCH, (1989 Mar 1) 46 (1) 131-6. Journal code: DBR; 8908639. ISSN: 0165-3806. Pub. country: Netherlands. Language: English.

AB By means of a **monoclonal antibody** (mab) against the rat liver nuclear L-T3 receptor (NT3R) and a **polyclonal** anti-GFAp serum, it has been possible to demonstrate nuclear **thyroid hormone receptors** in astrocyte cultures. On day 3, 47% of GFAp+ cell nuclei were labeled by 2B3 mab. Between day 3 and day 15, the number of GFA+ cell nuclei stained by 2B3 mab increased from 47 to 75%. Thyroid hormone nuclear receptors were present in fibrous and protoplasmic astrocytes. However, they developed asynchronously in both types of astrocytes. Indeed, 60% of fibrous astrocytes were stained by 2B3 mab on day 3 and this percentage reached 77% after 8 days in vitro. In contrast, only 30% of protoplasmic astrocytes were immunoreactive for 2B3 mab on day 3 and this percentage increased slowly reaching 47% on day 8 and around 75-80% on day 15. By immunoblotting, the **monoclonal antibody** recognized two bands of proteins with a molecular weight of 57 and 45 kDa respectively. These proteins have the same electrophoretic mobility as [125I]bromoacetyl-LT3 rat liver nuclear L-T3 receptor. This paper presents the first immunocytochemical localization of nuclear L-T3 receptors in astrocyte cultures. Furthermore, we show that **thyroid hormone receptors** develop more rapidly in fibrous than in protoplasmic astrocytes.

=> dup remove l12
PROCESSING COMPLETED FOR L12
L15 84 DUP REMOVE L12 (47 DUPLICATES REMOVED)

=> s l15 and humanized
L16 0 L15 AND HUMANIZED

=> s l15 and chimeric
L17 7 L15 AND CHIMERIC

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PROCESSING COMPLETED FOR L17
L18 7 DUP REMOVE L17 (0 DUPLICATES REMOVED)

=> d l18 1-7 cbib abs

L18 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2002 ACS
1995:761770 Document No. 123:162789 Ubiquitous nuclear receptors widely distributed in animal tissues and the genes encoding them and their uses. Liao, Shutsung; Song, Ching (Arch Development Corp., USA). PCT Int. Appl. WO 9513373 A1 19950518, 196 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US12883 19941108. PRIORITY: US 1993-152003 19931110.

AB Ubiquitous nuclear receptors (UR) found in many animal tissues are characterized and cDNAs encoding them cloned for use in the manuf. of the receptor polypeptides. Methods for using the receptor polypeptides in assays designed to select substances that interact with UR polypeptides for use in diagnostic, drug design and therapeutic applications are also discussed. A cDNA library from rat vagina in .lambda.ZAPII was screened with oligonucleotides derived from the conserved DNA-binding domains of the steroid/thyroid receptor superfamily. Hybridization studies found the transcript present in ventral prostate, seminal vesicle, testis, vagina, uterus, kidney, adrenal gland, liver, spleen, brain and heart. **Antibodies** raised against a fusion protein of the UR with the trpE gene product showed that the protein was localized in the nucleus of all the tissue tested. The receptor bound to perfect direct repeats of all hormone-responsive elements tested.

L18 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2002 ACS

1994:316754 Document No. 120:316754 A cDNA for a novel member of the steroid/**thyroid hormone receptor** family.
Kroczek, Richard; Mages, Hans Werner (Germany). PCT Int. Appl. WO 9404675 A2 19940303, 33 pp. DESIGNATED STATES: W: AU, BB, BG, BR, CA, CZ, FI, HU, JP, KP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1993-EP2223 19930819. PRIORITY: EP 1992-114134 19920819.

AB A cDNA for a novel member of the steroid/thyroid receptor family known as NOT (nuclear receptor of T-cells) is cloned and expressed. The cDNA and the protein and **antibodies** to the protein are useful in the treatment and diagnosis steroid receptor-related diseases (no data). A cDNA bank from activated human T-cells was screened for transcripts strongly upregulated by cell activation and 100 distinct cDNAs obtained. This pool was then screened for transcripts that were strongly transcribed at 2 h and 24 h post-activation and 5 such clones found. Partial sequences of one of these clones showed features typical of a steroid/thyroid receptor; the cDNA was used to screen a com. human placental genomic DNA library in .lambda.FIX to obtain an 11 kb insert. In situ hybridization showed the transcript to be abundant in fibroblasts, endothelial cells in synovial membranes and a subcompartment of spleen endothelial cells. Fragments of the protein were manufd. as fusion proteins with .beta.-galactosidase for the prepn. of antisera to the receptor that were used to demonstrate that the receptor was found in the nucleus and the cytoplasm.

L18 ANSWER 3 OF 7 SCISEARCH COPYRIGHT 2002 ISI (R)

94:114190 The Genuine Article (R) Number: MW036. ISOFORM-SPECIFIC INDUCTION OF A RETINOID-RESPONSIVE ANTIGEN AFTER BIOLISTIC TRANSFECTION OF **CHIMERIC** RETINOIC ACID **THYROID-HORMONE RECEPTORS** INTO A REGENERATING LIMB. PECORINO L T; LO D C; BROCKES J P (Reprint). LUDWIG INST CANC RES, 91 RIDING HOUSE ST, LONDON W1P 8BT, ENGLAND (Reprint); UNIV COLL LONDON, DEPT BIOCHEM & MOLEC BIOL, LONDON W1P 8BT, ENGLAND; LUDWIG INST CANC RES, LONDON W1P 8BT, ENGLAND. DEVELOPMENT (FEB 1994) Vol. 120, No. 2, pp. 325-333. ISSN: 0950-1991. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Retinoic acid (RA) induces secretory differentiation in the wound epidermis of a regenerating amphibian limb. We investigated the role of individual RA receptor (RAR) types in the newt wound epidermis by introducing chimaeric RA/thyroid hormone (T3) receptors (chi alpha 1 and chi delta 1) that can be activated by T3. A biolistic particle delivery system was employed to transfect cells in the wound epidermis of a regenerating limb and approximately 10% of the cells in targeted surface areas expressed marker genes. Both chi alpha 1 and chi delta 1 were comparable in their ability to stimulate transcription of a synthetic

reporter construct through a RA response element after activation with T3 in situ. This activation was also comparable to that obtained by the endogenous complement of RARs in the RA-treated, transfected wound epidermis. The RA-inducible WE3 antigen, a marker for secretory differentiation, which distinguishes the wound epidermis from normal skin (Tassava, R. A., Johnson-Wint, B. and Gross, J. 1986, J. Exp. Zool. 239, 229-240), was used to assess the functional role of chi alpha 1 and chi delta 1. Chimaeric receptors were transfected with an alkaline phosphatase marker gene, activated with T3, and the expression of both the marker and WE3 was analyzed by double-label immunofluorescence. Newt limbs transfected with chi delta 1 showed many double-labelled cells dependent on the presence of T3, whereas contralateral limbs transfected with an alkaline phosphatase marker lacking chimaeric receptor sequences did not. Limbs transfected with chi alpha 1 did not show double-labelled cells in the presence or absence of T3, whereas in an earlier study chi alpha 1, and not chi delta 1, inhibited growth. These results indicate that specific effects of RA can be mediated by particular types of RARs and demonstrate a novel approach for studying the action of RA on its target tissues.

L18 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2002 ACS

1993:75727 Document No. 118:75727 Hepatocyte nuclear factor 4 (HNF-4) and cloning of its cDNA. Sladek, Frances M.; Zhong, Weimin; Darnell, James E., Jr. (Rockefeller University, USA). PCT Int. Appl. WO 9211365 A1 19920709, 100 pp. DESIGNATED STATES: W: AU, CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US9733 19911223. PRIORITY: US 1990-631720 19901221.

AB DNA encoding HNF-4, cells producing HNF-4, methods of inhibiting HNF-4 function, and treatment of diseases by administering ligands for HNF-4 or apoCIII are claimed. The cDNA for rat liver HNF-4 was cloned and sequenced. HNF-4 has a structure analogous to the steroid/**thyroid hormones receptors**: it contains a zinc finger domain, and a hydrophobic C-terminus with similarity to the ligand binding domain of the other receptors. Also in the C-terminus is a proline-rich region characteristic of activator domains and possible phosphorylation sites. HNF-4 binds to its recognition site as a dimer. HNF-4 mRNA is present in liver, kidney, and intestine, but not in spleen, brain, white fat, lung, or heart. The factor binds to LF-A1 sites, but does not bind significantly to ERE, TRE, or GRE sites.

L18 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2002 ACS

1992:1748 Document No. 116:1748 Human thyroid-stimulating hormone receptor cDNA cloning and use in **monoclonal antibody** preparation. Milgrom, Edwin; Misrahi, Micheline; Loosfelt, Hugues; Atger, Michel (Institut National de la Sante et de la Recherche Medicale (INSERM), Fr.). PCT Int. Appl. WO 9110735 A2 19910725, 49 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (French). CODEN: PIXXD2. APPLICATION: WO 1991-FR25 19910115. PRIORITY: FR 1990-397 19900115.

AB A cDNA encoding the receptor for human TSH is cloned and expressed. The protein is used for the prepn. of **monoclonal** and polyclonal **antibodies** for use in the treatment of thyroid disorders (no data). The cDNA was cloned from a human thyroid cDNA bank in .lambda.gt10 by screening with porcine luteotropin/human chorionic gonadotropin receptor cDNA. Candidate clones were sequenced and confirmed by induction of thyroid hormone binding in COS cells. Transcription of the gene was limited to the thyroid gland. **Monoclonal antibodies** were prepd. against peptides from the hormone manufd. as fusion proteins with .beta.-galactosidase or with human ubiquitin in recombinant Escherichia coli.

L18 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2002 ACS

1991:529135 Document No. 115:129135 Cloning of androgen receptor and thyroid

receptor-like cDNAs. Liao, Shutsung; Chang, Chawnshang (Arch Development Corp., USA). PCT Int. Appl. WO 9107423 A1 19910530, 79 pp. DESIGNATED STATES: W: JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US6015 19901019. PRIORITY: US 1989-438775 19891117.

AB CDNAs encoding human steroid and thyroid hormone-like receptors are cloned and characterized and used as diagnostic reagents. CDNA libraries in .lambda.gt11 were screened with probes for the conserved DNA-binding region of the steroid-**thyroid hormone receptor** family. Candidates were then screened with probes specific for regions specific for other members of the family to exclude them. Clones that survived this were either "thyroid receptor-like" or "androgen receptor-like". Transcription/translation of androgen receptor-like cDNA in vitro indicated a mol. wt. of 79,000 for the protein. Competition studies confirmed that the translation product bound androgens specifically. The protein was immunopptd. with serum from prostate cancer patients. Expression for the manuf. of these proteins in prokaryotic and eukaryotic hosts and the prepn. of poly- and **monoclonal antibodies** are discussed.

L18 ANSWER 7 OF 7 MEDLINE

91242480 Document Number: 91242480. PubMed ID: 1645195. Retinoic acid receptor belongs to a subclass of nuclear receptors that do not form "docking" complexes with hsp90. Dalman F C; Sturzenbecker L J; Levin A A; Lucas D A; Perdew G H; Petkovitch M; Chambon P; Grippo J F; Pratt W B. (Department of Pharmacology, University of Michigan Medical School, Ann Arbor 48109.) BIOCHEMISTRY, (1991 Jun 4) 30 (22) 5605-8. Journal code: A0G; 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB We have recently reported that, in contrast to the glucocorticoid receptor, the **thyroid hormone receptor** does not bind to hsp90 when the receptor is translated in rabbit reticulocyte lysate [Dalman, F. C., Koenig, R. J., Perdew, G. H., Massa, E., & Pratt, W. B. (1990) J. Biol. Chem. 265, 3615-3618]. All of the steroid receptors that are known to bind hsp90 are recovered in the cytosolic fraction when hormone-free cells are ruptured in hypotonic buffer. In contrast, unliganded **thyroid hormone receptors** and retinoic acid receptors are tightly associated with nuclear components. In this paper, we translated the human estrogen receptor and the human retinoic acid receptor in reticulocyte lysate and then immunoadsorbed the [35S]methionine-labeled translation products with the 8D3 **monoclonal antibody** against hsp90. The estrogen receptor is bound to hsp90, as indicated by coimmunoadsorption, but the retinoic acid receptor is not. Translation and immunoadsorption of **chimeric** proteins containing the DNA binding domain of one receptor and the N-terminal and COOH-terminal segments of the other show that the DNA binding finger region of the estrogen receptor is neither necessary nor sufficient for hsp90 binding. These observations suggest that there are two classes within the steroid receptor family. In one class (e.g., glucocorticoid, mineralocorticoid, sex hormone, and dioxin receptors), the receptors bind to hsp90 and remain in some kind of inactive "docking" mode until hormone-triggered release of hsp90 occurs. In the retinoic acid/thyroid hormone class, the unligated receptors do not bind to hsp90, and the receptors appear to proceed directly to their high-affinity nuclear acceptor sites without entering the "docking" state.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 10:22:50 ON 08 MAY 2002

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L1      2302220 S ANTIBOD?
L2           42 S L1 AND "TRIPS"
L3            0 S L2 AND THYROID RECEPTOR INTERACTING PROTEINS
L4          417 S L1 AND CDC42
L5           61 S L4 AND MONOCLONAL
L6            1 S L5 AND POLYCLONAL
L7           41 DUP REMOVE L5 (20 DUPLICATES REMOVED)
L8            2 S L7 AND SH3
L9            2 DUP REMOVE L8 (0 DUPLICATES REMOVED)
L10          41 DUP REMOVE L5 (20 DUPLICATES REMOVED)
L11         631 S L1 AND THYROID HORMONE RECEPTOR
L12         131 S L11 AND MONOCLONAL
L13          14 S L12 AND POLYCLONAL
L14           6 DUP REMOVE L13 (8 DUPLICATES REMOVED)
L15          84 DUP REMOVE L12 (47 DUPLICATES REMOVED)
L16           0 S L15 AND HUMANIZED
L17           7 S L15 AND CHIMERIC
L18           7 DUP REMOVE L17 (0 DUPLICATES REMOVED)

=> d l15 1-84 cbib abs

L15  ANSWER 1 OF 84  SCISEARCH  COPYRIGHT 2002 ISI (R)
2002:249224   The Genuine Article (R) Number: 528EV. Novel alternatively
      spliced form of beta(3)-endonexin.  Fujimoto T T (Reprint); Katsutani S;
      Shimomura T; Fujimura K. Hiroshima Univ, Grad Sch Med, Dept Clin
      Pharmaceut Sci, Minami Ku, 1-2-3 Kasumi, Hiroshima 7348551, Japan
      (Reprint); Hiroshima Univ, Grad Sch Med, Dept Clin Pharmaceut Sci, Minami
      Ku, Hiroshima 7348551, Japan; Hiroshima Univ, Res Inst Radiat Biol & Med,
      Dept Hematol & Oncol, Minami Ku, Hiroshima 7348551, Japan. THROMBOSIS
      RESEARCH (1 JAN 2002) Vol. 105, No. 1, pp. 63-70. Publisher:
      PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON,
      OXFORD OX5 1GB, ENGLAND. ISSN: 0049-3848. Pub. country: Japan. Language:
      English.
      *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB       beta(3)-Endonexin is a binding protein to the cytoplasmic tail of
      beta(3) integrin and can activate alpha(IIb)beta(3) in Chinese hamster
      ovary (CHO) cells. Initially, two forms were identified, and only the
      shorter form showed the function. However, it localized mainly to the
      nucleus because of a nuclear localization signal ((KRKK)-R-62). We
      identified two additional forms of beta(3)-endonexin. One encoded 177
      amino acids and was identical to the protein previously reported as a
      thyroid hormone receptor-binding protein. The
      other is a novel shortest form encoding 62 amino acids. Although the novel
      form lacked nuclear localization signal and was observed diffusely in the
      cytoplasm of transfected cells, this form did not show interaction With 3
      integrin. Then, the ideal form as an integrin modulator was not found
      among these isoforms. Nevertheless, when the nuclear localization signal
      of the shorter form was disrupted, beta(3)-endonexin was localized near
      the cell surface and modulated the affinity of 01103 more intensively.
      These results suggest the presence of various isoforms and the
      relationship between subcellular localization and integrin-activating
      function of beta(3)-endonexin. (C) 2002 Elsevier Science Ltd. All rights
      reserved.

L15  ANSWER 2 OF 84  CAPLUS  COPYRIGHT 2002 ACS
2001:636361   Document No. 135:205530  Compositions and methods of use of HET,
      a novel modulator of estrogen action.  Oesterreich, Steffi; Osborne, C.
      K.; Lee, A. V.; Fuqua, S. A. (Board of Regents, the University of Texas
      System, USA).  PCT Int. Appl. WO 2001063292 A2 20010830, 140 pp.
      DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ,
      CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
      HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
      LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG,
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SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US6135 20010222. PRIORITY: US 2000-PV184097 20000222.

AB Disclosed are methods for the detection of tumor cells, in particular human breast cancer cells. Genetic and **antibody** probes and methods useful in detg. the presence of and monitoring tumor cell proliferation are also described. The methods involve detg. HET polypeptide expression, mRNA levels or loss of heterozygosity at human chromosomal locus 19p13 as a measure of tumor cell malignancy. These methods are also of use in distinguishing breast cancers that are resistant to estrogen antagonists, such as tamoxifen, from estrogen antagonist sensitive tumors. Also described are procedures for transforming cells with HET gene contg. vectors that express HET polypeptide. Such procedures may be of use in converting tamoxifen-resistant tumors into tamoxifen-sensitive tumors.

L15 ANSWER 3 OF 84 MEDLINE DUPLICATE 1

2001164856 Document Number: 21143912. PubMed ID: 11245686. Regulation of microglial development: a novel role for thyroid hormone. Lima F R; Gervais A; Colin C; Izembart M; Neto V M; Mallat M. (Institut National de la Sante et de la Recherche Medicale U.495, Hopital de la Salpetriere, 75651 Paris Cedex 13, France.) JOURNAL OF NEUROSCIENCE, (2001 Mar 15) 21 (6) 2028-38. Journal code: JDF; 8102140. ISSN: 1529-2401. Pub. country: United States. Language: English.

AB The postnatal development of rat microglia is marked by an important increase in the number of microglial cells and the growth of their ramified processes. We studied the role of thyroid hormone in microglial development. The distribution and morphology of microglial cells stained with isolectin B4 or **monoclonal antibody** ED1 were analyzed in cortical and subcortical forebrain regions of developing rats rendered hypothyroid by prenatal and postnatal treatment with methyl-thiouracil. Microglial processes were markedly less abundant in hypothyroid pups than in age-matched normal animals, from postnatal day 4 up to the end of the third postnatal week of life. A delay in process extension and a decrease in the density of microglial cell bodies, as shown by cell counts in the developing cingulate cortex of normal and hypothyroid animals, were responsible for these differences. Conversely, neonatal rat hyperthyroidism, induced by daily injections of 3,5,3'-triiodothyronine (T3), accelerated the extension of microglial processes and increased the density of cortical microglial cell bodies above physiological levels during the first postnatal week of life. Reverse transcription-PCR and immunological analyses indicated that cultured cortical ameboid microglial cells expressed the alpha1 and beta1 isoforms of nuclear **thyroid hormone receptors**. Consistent with the trophic and morphogenetic effects of thyroid hormone observed in situ, T3 favored the survival of cultured purified microglial cells and the growth of their processes. These results demonstrate that thyroid hormone promotes the growth and morphological differentiation of microglia during development.

L15 ANSWER 4 OF 84 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2001:277686 Document No.: PREV200100277686. RB18A, whose gene is localized on chromosome 17q12-q21.1, regulates in vivo p53 transactivating activity. Frade, Raymond (1); Balbo, Michelle (1); Barel, Monique (1). (1) INSERM U.354, Hopital Saint-Antoine, Centre INSERM, 184, rue du Fbg Saint-Antoine, Paris, 75012 France. FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A313. print. Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001 ISSN: 0892-6638. Language: English. Summary Language: English.

AB Among the different cellular factors which regulated p53 functions, we

previously identified RB18A, a new gene whose encoded 205 kDa protein interacted in vitro, through its C-terminal domain, with p53 (Drane et al., Oncogene, 1997). Therefore, we herein analyzed the in vivo role of RB18A by measuring its effect on p53 transactivating activity on physiological promoters. We demonstrated that: 1) in vivo RB18A interacted with wild type and mutant p53: indeed, both forms of p53 coprecipitated with RB18A immunoprecipitated on a **monoclonal** anti-RB18A **antibody** prepared in our laboratory; 2) RB18A regulated p53 transactivating activity on physiological promoters: indeed, RB18A activated Bax promoter and inhibited p21Wafl or IGF-BP3 promoters. FISH mapping led to localize RB18A gene on chromosome 17q12-q21.1. Among the genes already mapped on these two loci, some were associated to human cancers, as breast cancer, colorectal carcinoma and acute promyelocytic leukemia. These properties of RB18A should be related to its structural identity with two other components identified later than RB18A, i.e. TRAP220 and DRIP205, which belonged to the TRAP or DRIP complexes, constituted of at least 12 to 15 subunits, respectively. Indeed, TRAP220 and DRIP205 interacted with different hormone-activated nuclear receptors, as **thyroid hormone receptor**, vitamin D receptor, retinoic acid receptor alpha, retinoic X receptor alpha, peroxisome-proliferator-activated receptor (PPAR). Thus, RB18A (or TRAP220, DRIP205, PBP) by being a member of multiple-partner complexes and acting as a co-factor of transcriptional machinery may differently modulate different promoters. This is the first demonstration that RB18A/TRAP220/DRIP205, in a protein-protein interaction, in vivo regulates p53 transactivating activity.

L15 ANSWER 5 OF 84 CAPLUS COPYRIGHT 2002 ACS

2000:34974 Document No. 132:89248 Nucleic acid molecules encoding nuclear hormone receptor coactivators, and their diagnostic and therapeutic uses. Roeder, Robert G.; Fondell, Joseph D.; Xingyuan, Chao; Ito, Mitsuhiro (The Rockfeller University, USA). PCT Int. Appl. WO 2000001820 A2 20000113, 114 pp. DESIGNATED STATES: W: AU, CA, JP, MX; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US15052 19990701. PRIORITY: US 1998-110517 19980706.

AB Isolated cDNA mols. encoding human **thyroid hormone receptor**-assocd. proteins (TRAP220 and TRAP100) are provided. TRAPs are members of protein complexes that bind to nuclear hormone receptors in a ligand-dependent manner so that the receptor, upon activation by a corresponding hormone, regulates the transcription of a particular gene. The amino acid sequences of TRAP220 and TRAP100 share no obvious common motifs with other coactivators, except for the LXXLL motifs implicated in receptor-coactivator interactions. Also provided are methods of replicating and expressing such isolated nucleic acid mols., pharmaceutical compns. comprising TRAPs, and methods of modulating gene expression via administration of therapeutically effective amts. of such pharmaceutical compns.

L15 ANSWER 6 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

2000:601570 The Genuine Article (R) Number: 340NX. Basic helix-loop-helix proteins and the timing of oligodendrocyte differentiation. Kondo T (Reprint); Raff M. UNIV COLL LONDON, MRC, DEV NEUROBIOL PROGRAMME, MOL CELL BIOL LAB, MORTIMER ST, LONDON WC1E 6BT, ENGLAND (Reprint); UNIV COLL LONDON, DEPT BIOL, LONDON WC1E 6BT, ENGLAND. DEVELOPMENT (JUL 2000) Vol. 127, No. 14, pp. 2989-2998. Publisher: COMPANY OF BIOLOGISTS LTD. BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND. ISSN: 0950-1991. Pub. country: ENGLAND. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB An intracellular timer in oligodendrocyte precursor cells is thought to help control the timing of their differentiation. We show here that the expression of the Hes5 and Mash1 genes, which encode neural-specific bHLH proteins, decrease and increase, respectively, in these cells with a time

course expected if the proteins are part of the timer. We show that enforced expression of Hes5 in purified precursor cells strongly inhibits the normal increase in the **thyroid hormone receptor** protein TR beta 1, which is thought to be part of the timing mechanism; it also strongly inhibits the differentiation induced by either mitogen withdrawal or thyroid hormone treatment. Enforced expression of Mash1, bg contrast, somewhat accelerates the increase in TR beta 1 protein. These findings suggest that Hes5 and Mash1 may be part of the cell-intrinsic timer in the precursor cells.

L15 ANSWER 7 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2000150023 EMBASE **Thyroid hormone receptor** .beta.1 is expressed in the human hair follicle. Billoni N.; Buan B.; Gautier B.; Gaillard O.; Mahe Y.F.; Bernard B.A.. N. Billoni, Life Sciences, L'OREAL Advanced Res. Laboratories, L'OREAL Hair Biology Group, 90 rue du general Roguet, 92583 Clichy Cedex, France. British Journal of Dermatology 142/4 (645-652) 2000.
Refs: 31.
ISSN: 0007-0963. CODEN: BJDEAZ. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB To understand better the mechanisms by which thyroid hormone can exert its effects on the hair follicle, we looked for the expression of members of the **thyroid hormone receptor** (TR) family in human hair follicles. Immunoreactive TRs were detected in both dermal and epithelial compartments of the human pilosebaceous unit. Using reverse transcriptase-polymerase chain reaction, we established that TR.beta.1 was the predominant form of TR expressed in the human hair follicle. In addition, we investigated the effects of 3,3',5-triiodo-L-thyronine (T3) on the survival of human hair follicles in vitro, to understand the role of this thyroid hormone on hair follicle homeostasis. A physiological level of free T3 significantly enhanced human hair survival in vitro.

L15 ANSWER 8 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2000150020 EMBASE Thyroid hormone and hair growth. Messenger A.G.. Dr. A.G. Messenger, Royal Hallamshire Hospital, Department of Dermatology, Glossop Road, Sheffield S10 2JF, United Kingdom. a.g.messenger@sheffied.ac.uk. British Journal of Dermatology 142/4 (633-634) 2000.
Refs: 9.
ISSN: 0007-0963. CODEN: BJDEAZ. Pub. Country: United Kingdom. Language: English.

L15 ANSWER 9 OF 84 CAPLUS COPYRIGHT 2002 ACS
1999:375698 Document No. 131:28610 Single-chain **monoclonal antibody** fusion reagents that regulate transcription in vivo and methods of screening for such scFv's. Hoeffler, James P.; Russell, Marijane (Invitrogen Corporation, USA). PCT Int. Appl. WO 9928502 A1 19990610, 132 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US21407 19971128.
AB A method of screening a DNA construct library for a single chain **monoclonal antibody** fusion reagent capable of binding a transcription-assocd. biomol. in vivo is described. The method, a variant of the yeast two-hybrid system, comprises fusing an antigenic portion of a transcription-assocd. biomol. (e.g., CREB or ATF-2) to a DNA-binding domain (DBD), such as the DBD of the LexA protein, to provide a bait protein. The scFv library is fused to a transactivating domain, e.g., the transactivating domain of VP16. The ability of scFv's to bind to the bait antigen is assayed in recombinant Saccharomyces cerevisiae contg. a reporter gene (lacZ, HIS3) controlled by a LexA-binding UAS. Single chain **monoclonal antibody** fusion reagents capable of binding transcriptional assocd. biomols. in vivo are provided. Single chain **monoclonal antibody** fusion reagents which are capable of regulating transcription in vivo are also provided. Therapeutic methods

for regulating the transcription of a gene $i(in vivo) are also described. A method is further provided for screening a plurality of compds. for specific binding affinity with a single chain **monoclonal antibody** fusion reagent. A method is also described for diagnosing a physiol. disorder manifested by an abnormal level of a transcription assocd. biomol. A DNA construct (pVP16Zeo) as well as primers for the construction and screening of single chain **monoclonal antibody** fusion reagent libraries to facilitate the isolation and prodn. of single chain **monoclonal antibody** fusion reagents in yeast and E.coli are also provided. A kit for screening a DNA construct library for a single chain **monoclonal antibody** fusion reagent capable of binding a transcriptional assocd. biomol. in vivo is also provided.

L15 ANSWER 10 OF 84 CAPLUS COPYRIGHT 2002 ACS

1999:707902 Document No. 131:321536 Indirect antigen immobilization and method for autoantibody detection. Igarashi, Koji (Tosoh Corp., Japan). Jpn. Kokai Tokkyo Koho JP 11304801 A2 19991105 Heisei, 6 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1998-107439 19980417.

AB Insol. carrier-immobilized antigen is prepd. for immunoassay of autoantibody and for diagnosis of autoimmune diseases. The antigen is selected from **thyroid hormone receptor** or glutamate decarboxylase.

L15 ANSWER 11 OF 84 CAPLUS COPYRIGHT 2002 ACS

1999:367416 Document No. 131:139767 Differential regulation of direct repeat 3 vitamin D3 and direct repeat 4 thyroid hormone signaling pathways by the human TR4 orphan receptor. Lee, Yi-Fen; Young, Win-Jing; Lin, Wen-Jye; Shyr, Chih-Rong; Chang, Chawnshang (George Whipple Laboratory for Cancer Research, Department of Pathology, Urology, Radiation Oncology, University of Rochester Medical Center, Rochester, NY, 14642, USA). Journal of Biological Chemistry, 274(23), 16198-16205 (English) 1999. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB In situ hybridization anal. demonstrated that abundant testicular orphan receptor (TR4) transcripts were detected in kidney, intestine, and bone, which are vitamin D3 target organs. Cell transfection studies also demonstrated that the expression of the vitamin D3 target gene, 25-hydroxyvitamin D3 24-hydroxylase, can be repressed by TR4 through high affinity binding (Kd = 1.32 nM) to the direct repeat 3 vitamin D3 receptor response element (DR3VDRE). This TR4-mediated repression of DR3VDRE is in contrast to the authors' earlier report that TR4 could induce thyroid hormone target genes contg. a direct repeat 4 thyroid hormone response element (DR4T3RE). Electrophoretic mobility shift assay using several TR4 **monoclonal antibodies** when combined with either TR4-DR3VDRE or TR4-DR4T3RE showed a distinct supershifted pattern, and proteolytic anal. further demonstrated distinct digested peptides with either TR4-DR3VDRE or TR4-DR4T3RE. These results may therefore suggest that TR4 can adapt to different conformations once bound to DR3VDRE or DR4T3RE. The consequence of these different conformations of TR4-DR3VDRE and TR4-DR4T3RE may allow each of them to recruit different coregulators. The differential repression of TR4-mediated DR3VDRE and DR4T3RE transactivation by the receptor interacting protein 140, a TR4 coregulator, further strengthens the authors' hypothesis that the specificity of gene regulation by TR4 can be modulated by protein-DNA and protein-protein interactions.

L15 ANSWER 12 OF 84 CAPLUS COPYRIGHT 2002 ACS

1999:766664 Document No. 132:102964 Interaction between nuclear hormone receptors and coactivators analyzed using a nonradioactive "pull-down" assay. Bakker, O.; van Beeren, H. C.; Emrich, T.; Holtke, H. -J.; Wiersinga, W. M. (Endocrinology, F5-171, Academic Medical Centre, Amsterdam, 1105 AZ, Neth.). Analytical Biochemistry, 276(1), 105-106

(English) 1999. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Academic Press.

AB Many transcription factors signal their presence to the transcription initiation complex via protein-protein interactions. Among these factors are the family of the nuclear hormone receptors which repress transcription by interaction with corepressors like N-CoR and SMRT and increase transcription via coactivators like SRC-1, and GRIP-1. The interaction between these proteins can be studied in vitro by binding one of them to a support which can be easily sepd. from the incubation mixt. (usually glutathione-Sepharose beads) and labeling the other so that it can be detected after electrophoretic sepn. (usually 35S label). The authors describe a novel nonradioactive method for studying these protein-protein interactions (pull-down assay) using the interaction between the **thyroid hormone receptor** .beta.1 (TR.beta.1) and the nuclear receptor interaction domain (NID) of the coactivator GRIP-1 as a model. The required sensitivity is reached by using a hemagglutinin (HA) tag and a high-affinity **monoclonal** anti-hemagglutinin **antibody** (clone 3F10) conjugated with peroxidase in combination with a long-acting peroxidase substrate. (c) 1999 Academic Press.

L15 ANSWER 13 OF 84 CAPLUS COPYRIGHT 2002 ACS

1998:427690 Document No. 129:92583 Assays for functional nuclear receptors. Nargessi, Ruhangiz Dokhi (Chiron Diagnostics Corp., USA). U.S. US 5770176 A 19980623, 28 pp. (English). CODEN: USXXAM. APPLICATION: US 1995-569977 19951208.

AB Methods and test kits for detecting, or detecting and quantitating functional nuclear receptors in cell or tissue samples are disclosed. Such methods provide highly sensitive assays requiring small sample sizes and short turnaround times. The methods are useful in developing prognoses and/or treatment programs for cancer patients, esp. in detg. whether therapy to interfere with the receptor's activation of gene transcription, such as, endocrine therapy, would be helpful. An estrogen receptor (ER) ELISA involved incubating assay buffer, estradiol soln., and patient cytosol or controls in ER **monoclonal antibody** -coated microtiter wells; washing the wells; and adding an estrogen response element octamer that had been biotinylated. After incubation and washing, the wells were reacted with streptavidin-horseradish peroxidase conjugate. TMB was the enzyme substrate used in the assay. The absorbance was measured at 450 nm. Twenty-three breast cancer cytosols were assayed.

L15 ANSWER 14 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 2

1998072147 EMBASE 3,5-Diiodothyronine binds to subunit Va of cytochrome-c oxidase and abolishes the allosteric inhibition of respiration by ATP. Arnold S.; Goglia F.; Kadenbach B.. B. Kadenbach, Fachbereich Chemie, Philipps-Universitat, Hans-Meerwein-Strasse, D-35032 Marburg, Germany. Kadenbach@chemie.uni-marburg.de. European Journal of Biochemistry 252/2 (325-330) 1 Mar 1998.
Refs: 43.
ISSN: 0014-2956. CODEN: EJBCAI. Pub. Country: Germany. Language: English. Summary Language: English.

AB The short-term effects of thyroid hormones, which do not occur via gene expression, were postulated to be based on interaction of diiodothyronines with mitochondria. We demonstrate specific binding of labelled 3,5-diiodothyronine to subunit Va of cytochrome-c oxidase from bovine heart. 3,5-Diiodothyronine, and to a small extent triiodothyronine, but not thyroxine and thyronine, abolish the allosteric inhibition of ascorbate respiration of reconstituted cytochrome c oxidase by ATP [Arnold, S. and Kadenbach, B. (1997) Eur. J. Biochem. 249, 350-354]. This abolition of ATP-inhibition by 3,5-diiodothyronine is completely prevented by a **monoclonal antibody** to subunit Va. The results explain at the molecular level the short-term action of thyroid hormones

on basal metabolic rate.

L15 ANSWER 15 OF 84 MEDLINE DUPLICATE 3
1998260097 Document Number: 98260097. PubMed ID: 9597806.
Immunohistochemical localization of thyroid hormone nuclear receptors in human hair follicles and in vitro effect of L-triiodothyronine on cultured cells of hair follicles and skin. Ahsan M K; Urano Y; Kato S; Oura H; Arase S. (Department of Dermatology, University of Tokushima School of Medicine, Japan.) JOURNAL OF MEDICAL INVESTIGATION, (1998 Feb) 44 (3-4) 179-84. Journal code: CXV; 9716841. ISSN: 1343-1420. Pub. country: Japan. Language: English.

AB To investigate the cellular basis of the action of thyroid hormone on hair follicles, we studied the immunohistochemical localization of **thyroid hormone receptors** (TRs) in human scalp skin using a mouse **monoclonal antibody**, TR alpha 1 (C4) against TRs. Immunoreactive TRs were detected in the nuclei of the outer root sheath cells (ORSCs), dermal papilla cells (DPCs), fibrous sheath cells of hair follicles, hair arrector pili muscle cells and sebaceous gland cells. However, nuclei of hair matrix cells were not clearly stained with TR alpha 1 (C4). The epidermis showed positive nuclear staining by the **antibody**. Ductal and secretory portions of eccrine sweat glands were also stained with the **antibody** as we had expected. In the dermis, almost all the cell components including fibroblasts, vascular endothelial and smooth muscle cells, and Schwann cells were positively stained. Immunofluorescence also showed TRs expression in cultured ORSCs, DPCs, epidermal keratinocytes and dermal fibroblasts. L-triiodothyronine stimulated the proliferation and/or metabolism of all these four types of cells significantly, although there was variation at the rate of stimulation. Whereas, structurally similar, but metabolically inactive analog, reverse T3 had no effect. These results demonstrate the presence of thyroid hormone nuclear receptors in human hair follicles. Furthermore, the presence of TRs in different cell types in the skin suggests numerous direct effects of thyroid hormone on this target tissue.

L15 ANSWER 16 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:404683 The Genuine Article (R) Number: ZP629. Cell-intrinsic timers and thyroid hormone regulate the probability of cell-cycle withdrawal and differentiation of oligodendrocyte precursor cells. Gao F B; Apperly J; Raff M (Reprint). UNIV LONDON UNIV COLL, MRC, MOL CELL BIOL LAB, DEV NEUROBIOL PROGRAMME, LONDON WC1E 6BT, ENGLAND (Reprint); UNIV LONDON UNIV COLL, MRC, MOL CELL BIOL LAB, DEV NEUROBIOL PROGRAMME, LONDON WC1E 6BT, ENGLAND; UNIV LONDON UNIV COLL, DEPT BIOL, LONDON WC1E 6BT, ENGLAND. DEVELOPMENTAL BIOLOGY (1 MAY 1998) Vol. 197, No. 1, pp. 54-66. Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0012-1606. Pub. country: ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB During vertebrate development many types of precursor cells divide a limited number of times before they stop dividing and terminally differentiate. It is unclear what causes the cells to stop dividing when they do. We have been studying this problem in the oligodendrocyte cell Lineage, which is responsible for myelination in the vertebrate central nervous system. Here we show for the first time that in clonal cultures of oligodendrocyte precursor cells purified from embryonic day 18 (E18) rat optic nerves the first oligodendrocytes develop within 3-4 days, equivalent to the time they first differentiate in the nerve, and that this timely differentiation depends on the presence of thyroid hormone. These findings suggest that a cell-intrinsic, thyroid-hormone-regulated timer determines when the first oligodendrocytes develop. Whereas the first oligodendrocytes develop asynchronously within clones, the vast majority develop after the first week in culture and do so more synchronously within clones. We show that beta 1 **thyroid hormone receptors** in the precursor cells increase in

clonal cultures in the absence of thyroid hormone in parallel with the increasing sensitivity of the cells to the cell-cycle-arresting activity of thyroid hormone; moreover, the increase in beta 1 receptors, like the timer itself, is accelerated at 33 degrees C compared to 37 degrees C, suggesting that the increase in receptors may be part of the intrinsic timer. Finally, we shaw that the precursor cells do not divide Indefinitely when stimulated to divide extensively in the absence of thyroid hormone but, instead, eventually stop dividing and either die or differentiate. (C) 1998 Academic Press.

L15 ANSWER 17 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:289868 The Genuine Article (R) Number: BK65Z. Gene expression during amphibian limb regeneration. Geraudie J (Reprint); Ferretti P. UNIV PARIS 07, DEV BIOL LAB, CASE 7077, F-75251 PARIS 05, FRANCE (Reprint); UCL, INST CHILD HLTH, DEV BIOL UNIT, LONDON WC1N 1EH, ENGLAND. INTERNATIONAL REVIEW OF CYTOLOGY-A SURVEY OF CELL BIOLOGY (FEB 1998) Vol. 180, pp. 1-50. Publisher: ACADEMIC PRESS INC. 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0074-7696. Pub. country: FRANCE; ENGLAND. Language: English.

L15 ANSWER 18 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
97:843759 The Genuine Article (R) Number: YF219. The glucocorticoid receptor is associated with the RNA-binding nuclear matrix protein hnRNP U. Eggert M; Michel J; Schneider S; Bornfleth H; Baniahmad A; Fackelmayer F O; Schmidt S; Renkawitz R (Reprint). UNIV GIESSEN, GENET INST, HEINRICH BUFF RING 58-62, D-35392 GIESSEN, GERMANY (Reprint); UNIV GIESSEN, GENET INST, D-35392 GIESSEN, GERMANY; UNIV HEIDELBERG, INST ANGEW PHYS, D-69120 HEIDELBERG, GERMANY; UNIV CONSTANCE, FAK BIOL, D-78646 CONSTANCE, GERMANY. JOURNAL OF BIOLOGICAL CHEMISTRY (7 NOV 1997) Vol. 272, No. 45, pp. 28471-28478. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0021-9258. Pub. country: GERMANY . Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The glucocorticoid receptor (GR) is a ligand-dependent transcription factor that is able to modulate gene activity by binding to its response element, interacting with other transcription factors, and contacting several accessory proteins such as coactivators. Here we show that GRIP120, one of the factors we have identified to interact with the glucocorticoid receptor, is identical to the heterogeneous nuclear ribonucleoprotein U (hnRNP U), a nuclear matrix protein binding to RNA as well as to scaffold attachment regions, GR hnRNP U complexes were identified by blotting and coimmunoprecipitation. The subnuclear distribution of GR and hnRNP U was characterized by indirect immunofluorescent labeling and confocal laser microscopy demonstrating a colocalization of both proteins, Using a nuclear transport-deficient deletion of hnRNP U, nuclear translocation was seen to be dependent on GR and dexamethasone. Transient transfections were used to identify possible interaction domains, Overexpressed hnRNP U interfered with glucocorticoid induction, and the COOH-terminal domains of both proteins were sufficient in mediating the transcriptional interference, A possible functional role for this GR binding-protein in addition to its binding to the nuclear matrix, to RNA, and to scaffold attachment regions is discussed.

L15 ANSWER 19 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 4
97120348 EMBASE Document No.: 1997120348. Critical role of glutamine 252 in the hormone-dependent transcriptional activity of the thyroid hormone .beta.1 nuclear receptor. Bhat M.K.; McPhie P.; Cheng S.-Y.. S.-Y. Cheng, Building 37, 37 Convent Dr., Bethesda, MD 20892-4255, United States. sycheng@helix.nih.gov. Biochemistry 36/14 (4233-4239) 1997.
Refs: 37.
ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.
AB To understand the molecular basis of the ligand-dependent transcriptional

activity of thyroid hormone nuclear receptors (TRs), we investigated the effect of mutation of glutamine 252 (Q252) on the function of human TR subtype .beta.1 (wTR.beta.1). Q252 is conserved in TRs in all species and is located in a region of the hormone binding domain that has been shown to undergo 3,3',5-triiodo-L-thyronine (T3) induced conformational changes. Q252 was mutated to Gly (Q252G) or Asn (Q252N) and their immunoreactivity, hormone, and DNA binding activities were characterized. Mutants Q252G and Q252N bound to T3 with similar affinity as the wTR.beta.1. However, they failed to interact with a **monoclonal** anti-wTR.beta.1 **antibody** whose epitope is located in the region of amino acids 248-256, suggesting that mutation of Q252 to Gly or Asn resulted in local structural alteration in TR.beta.1. In addition, mutation of Glu to Gly or Asn led to increases in their binding to the thyroid hormone response elements (TREs) as homodimers and as heterodimers with the retinoid X receptor. Mutants Q252G and Q252N were more effective as repressors in the absence of T3, while both had a 1.5-2-fold higher T3- dependent transcriptional activity mediated by three TREs than the wTR.beta.1. The increases in the transcriptional activity were not due to an increase in the expression of the mutant receptor proteins because the in vivo expression level of the mutant receptor proteins was identical to that of the wTR.beta.1. Our data indicate that the T3-dependent transcriptional activity is not entirely dependent on the T3 binding activity of the receptor. The interplay of ligand and DNA binding domains plays a pivotal role in the transcriptional activity of the TRs.

L15 ANSWER 20 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
97054697 EMBASE Document No.: 1997054697. Tissue-specific stabilization of the thyroid hormone .beta.1 nuclear receptor by phosphorylation. Ting Y.-T.; Bhat M.K.; Wong R.; Cheng S.-Y.. S.-Y. Cheng, Laboratory of Molecular Biology, NCI, National Institutes of Health, 37 Convent Dr., Bethesda, MD 20892-4255, United States. Journal of Biological Chemistry 272/7 (4129-4134) 1997.
Refs: 32.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB The present study evaluated the expression and regulation of endogenous **thyroid hormone receptors** (TRs) in cultured cells. In COS-1 cells, the endogenous TR, subtype .beta.1 (TR.beta.1), but not subtype .beta.2 or .alpha.1, was induced to express by okadaic acid (OA) in a concentration-dependent manner. The induced TR.beta.1 had immunoreactivity and partial V8 proteolytic maps similar to those of the transfected and in vitro translated human TR.beta.1 (h-TR.beta.1). The OA-induced expression of endogenous TR.beta.1 was, however, not observed in a variety of other cultured cell lines tested, indicating that the induction was cell type-dependent. TR.beta.1 induced by OA was a multisite phosphorylated protein, in which serine and threonine in a ratio of 10:1 were phosphorylated. The induced TR.beta.1 was functional as it could mediate the thyroid hormone-dependent transcriptional activity via several thyroid hormone response elements. The induction of endogenous TR.beta.1 expression by OA was not accompanied by an increase in mRNA levels but was the result of an increase in the stability of the TR.beta.1 protein. This is the first report to indicate that one of the mechanisms by which the TR isoforms are differentially expressed is via the tissue-specific stabilization of the TR isoform proteins. Furthermore, this selective stability of TR.beta.1 could be conferred by phosphorylation.

L15 ANSWER 21 OF 84 MEDLINE DUPLICATE 5
97271687 Document Number: 97271687. PubMed ID: 9126603. One-step immunoaffinity purification of recombinant human retinoic acid receptor gamma. Repa J J; Berg J A; Kaiser M E; Hanson K K; Strugnell S A; Clagett-Dame M. (Interdepartmental Graduate Program in Nutritional Sciences, University of Wisconsin-Madison 53706, USA.) PROTEIN EXPRESSION AND PURIFICATION, (1997 Apr) 9 (3) 319-30. Journal code: BJV; 9101496.

ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Retinoic acid receptors (RAR) are members of the steroid/**thyroid hormone receptor** superfamily and serve as ligand-activated transcription factors. In order to facilitate studies of receptor protein, we have generated a **monoclonal antibody** to the human RAR gamma, and have developed a procedure to purify the full-length receptor expressed in insect cells. The **monoclonal antibody** (A10) was developed using as antigen a carboxy-terminal fragment of the human RAR gamma expressed as a bacterial fusion protein. The A10 **monoclonal antibody** binds to both native and denatured forms of the human RAR gamma. This **antibody** was immobilized on a resin and used to purify full-length, baculovirus-expressed human RAR gamma to near homogeneity. The immunoaffinity-purified receptor is > 90-95% pure as revealed by silver-stained gels. The identity of the single protein band as RAR gamma was verified by immunoblotting using a polyclonal **antibody** to an epitope distinct from that recognized by the A10 **antibody**. The pure human RAR gamma is functional with respect to both ligand and DNA binding. Scatchard analysis of 3H-labeled all-trans retinoic acid binding to purified human RAR gamma revealed a single, high-affinity binding site with a Kd of approximately .2 nM. Binding of the pure RAR gamma to a DR5-type retinoic acid response element was also studied. Response element binding by RAR gamma required the presence of the retinoid X receptor, but did not require the presence of additional proteins. Human RAR gamma protein purified in this fashion will be useful in future structural and functional studies.

L15 ANSWER 22 OF 84 MEDLINE DUPLICATE 6

97371680 Document Number: 97371680. PubMed ID: 9227894. An immunocytochemical analysis of the expression of **thyroid hormone receptor** alpha and beta proteins during natural and thyroid hormone-induced metamorphosis in Xenopus. Fairclough L; Tata J R. (Division of Developmental Biochemistry, MRC National Institute for Medical Research, London, UK.) DEVELOPMENT GROWTH AND DIFFERENTIATION, (1997 Jun) 39 (3) 273-83. Journal code: E7Y; 0356504. ISSN: 0012-1592. Pub. country: Japan. Language: English.

AB Amphibian metamorphosis is characterized by the upregulation of **thyroid hormone receptor** (TR) mRNA in all tissues of tadpole during both the natural and thyroid hormone (TH)-induced development. The two TR genes, termed alpha and beta, are members of a large multigene family of nuclear receptors related to the cellular homolog of the oncogene c-erbA. The phenomenon of upregulation is more marked for the beta than the alpha isoform. To determine whether or not the auto-induction of the transcripts is paralleled by that of TR proteins, non-cross-reacting **monoclonal antibodies** were prepared against Xenopus laevis TR alpha and beta (xTR alpha, beta) in order to analyze immunocytochemically their expression and localization. Three tadpole tissues that exemplify three major consequences of gene re-programing during natural and TH-induced metamorphosis were studied: (i) Liver that undergoes extensive functional switching; (ii) small intestinal epithelium that exhibits substantial cell death prior to major structural and biochemical modifications; and (iii) hind limb-bud as an example of de novo morphogenesis. It was shown that xTR alpha protein is generally more abundant in these tissues, and its expression is developmentally and hormonally less regulated, than is xTR beta. The auto-induction of xTR beta was particularly intense at 5 days after administration of triiodo-thyronine (T3) to both pre-metamorphic (stage 52) tadpoles and at the onset of natural metamorphosis (stage 55). In the developing hind limb-bud at both stages the upregulation of TR beta is topologically restricted, being particularly intense in dense pockets of cells, presumably rich in chondrocytes. It was concluded that the distribution and expression of xTR alpha and beta proteins match partially, but not fully, those of their transcripts during natural and

hormone-induced metamorphosis.

L15 ANSWER 23 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
97:407406 The Genuine Article (R) Number: WZ916. Promoter- and cell-specific responses to sex steroids. Milgrom E (Reprint); Savouret J F; Mantel A; PerrotApplanat M; Delabre K; Lescop P. HOP BICETRE, INSERM, U135, UNITE RECH, 78 RUE GEN LECLERC, F-94295 LE KREMLIN BICETR, FRANCE (Reprint). OSTEOPOROSIS INTERNATIONAL (MAY 1997) Vol. 7, Supp. [1], pp. 23-28. Publisher: SPRINGER-VERLAG LONDON LTD. SWEETAPPLE HOUSE CATTESHALL ROAD, GODALMING, SURREY, ENGLAND GU7 3DJ. ISSN: 0937-941X. Pub. country: FRANCE. Language: English.

L15 ANSWER 24 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
96137418 EMBASE Document No.: 1996137418. Suppression of the human erythropoietin gene expression by the TR2 orphan receptor, a member of the steroid receptor superfamily. Lee H.-J.; Young W.-J.; Shih C.C.-Y.; Chang C.. Comprehensive Cancer Center, University of Wisconsin, 600 Highland Ave.,Madison, WI 53792, United States. Journal of Biological Chemistry 271/17 (10405-10412) 1996.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.
AB A DNA response element, TR2RE-EPO (5'-TCTGAC-CTCTCGACCTAC-3') has been identified in the 3'-minimal hypoxia-inducible enhancer of the human erythropoietin gene for the TR2 orphan receptor, an androgen-repressed transcription factor and a member of the steroid/**thyroid hormone receptor** superfamily. Electrophoretic mobility shift assay showed a specific binding with high affinity (K(d) = 0.14 nM) between the TR2 orphan receptor and the TR2RE-EPO. Our data further indicated that this specific binding is not due to the homo-dimerization of the TR2 orphan receptor. In addition, reporter gene expression using chloramphenicol acetyltransferase assay demonstrated that the TR2 orphan receptor may suppress the expression of the chloramphenicol acetyltransferase activities via the TR2RE-EPO in the hypoxic/normoxic human hepatoma HepG2 cells. Finally, our in situ hybridization data also indicated that the TR2 orphan receptor and the erythropoietin transcripts can be co-expressed in mouse kidney and liver. Together, our data suggest that the human erythropoietin gene could represent the first human target gene regulated directly by the human TR2 orphan receptor.

L15 ANSWER 25 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
96:654579 The Genuine Article (R) Number: VE441. IS RETINOIC ACID AN ENDOGENOUS LIGAND DURING URODELE LIMB REGENERATION. VIVIANO C M; BROCKES J P (Reprint). UNIV COLL LONDON, LUDWIG INST CANC RES, 91 RIDING HOUSE ST, LONDON W1P 8BT, ENGLAND (Reprint); UNIV COLL LONDON, LUDWIG INST CANC RES, LONDON W1P 8BT, ENGLAND; UNIV COLL LONDON, DEPT BIOCHEM & MOL BIOL, LONDON W1P 8BT, ENGLAND. INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY (AUG 1996) Vol. 40, No. 4, pp. 817-822. ISSN: 0214-6282. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The effects of retinoids on a regenerating urodele limb make them interesting candidates for endogenous ligands during regeneration. We review the evidence for considering this possibility. This includes analysis of retinoids and retinoic acid receptors in the regenerate, and studies on activation of retinoid reporter genes. Recent work has provided evidence that the wound epidermis is a source of 9-cis retinoic acid, and may be a favorable model for studying the synthesis and release of this modulator.

L15 ANSWER 26 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
96:428892 The Genuine Article (R) Number: UN421. THE ISOFORM-SPECIFIC EXPRESSION OF THE TRIIODOTHYRONINE RECEPTOR IN OSTEOBLASTS AND OSTEOCLASTS . ALLAIN T J; YEN P M; FLANAGAN A M; MCGREGOR A M (Reprint). UNIV LONDON KINGS COLL, SCH MED & DENT, DEPT MED, BESSEMER RD, LONDON SE5 9PJ, ENGLAND

(Reprint); UNIV LONDON KINGS COLL, SCH MED & DENT, DEPT MED, LONDON SE5 9PJ, ENGLAND; BRIGHAM & WOMENS HOSP, GENET MOLEC LAB, BOSTON, MA, 02115; ST MARYS HOSP, SCH MED, DEPT HISTOPATHOL, LONDON W2 1PG, ENGLAND. EUROPEAN JOURNAL OF CLINICAL INVESTIGATION (MAY 1996) Vol. 26, No. 5, pp. 418-425. ISSN: 0014-2972. Pub. country: ENGLAND; USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Hyperthyroidism is associated with an increase in both osteoblast and osteoclast activity. We have previously shown that, in vitro, osteoclasts do not respond directly to tri-iodothyronine to increase bone resorption but that the effect is mediated by another bone cell, probably the osteoblast. To investigate this issue further we have studied the isoform-specific expression of thyroid receptor (TR) protein in human osteoclasts derived from an osteoclastoma (giant cell tumour of bone) and the expression of TR mRNA and protein in the osteoblastic cell lines MG 63 and UMR 106. Three major TR receptor variants have been described; TR alpha 1 and TR beta are functional receptors whereas c-erbA alpha 2 is a non-functional variant. Northern blot analysis using [P-32]- cDNA probes against human TR alpha 1, c-erbA alpha 2 and TR beta demonstrated specific binding of these probes to mRNA from MG 63 and UMR 106. mRNA for all three receptor variants was observed in both cell lines, in UMR 106 multiple mRNA transcripts were present for TR alpha 1 and TR beta. Immunohistochemical staining with **antibodies** recognizing a common TR alpha epitope and specific c-erbA alpha 2 and TR beta epitopes extended these observations by demonstrating receptor protein in both osteoblasts and osteoclasts. These findings are consistent with previous observations of TR expression in osteoblast-like cells and are the first direct demonstration of TR in osteoclasts.

L15 ANSWER 27 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
97:105309 The Genuine Article (R) Number: WD950. Stability of the ligand estrogen receptor interaction depends on estrogen response element flanking sequences and cellular factors. Anolik J H; Klinge C M (Reprint); Brolly C L; Bambara R A; Hilf R. UNIV LOUISVILLE, SCH MED, DEPT BIOCHEM, LOUISVILLE, KY 40292 (Reprint); UNIV ROCHESTER, SCH MED & DENT, DEPT BIOCHEM, ROCHESTER, NY 14642; UNIV LOUISVILLE, SCH MED, CTR CANC, LOUISVILLE, KY 40292. JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY (DEC 1996) Vol. 59, No. 5-6, pp. 413-429. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB. ISSN: 0960-0760. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To determine whether accessory proteins mediate the ligand- and DNA sequence-dependent specificity of estrogen receptor (ER) interaction with DNA, the binding of partly purified vs highly purified bovine ER to various estrogen response elements (EREs) was measured in the presence of different ER ligands. Partly purified estradiol-liganded ER (E(2)-ER) binds cooperatively to stereoaligned tandem EREs flanked by naturally occurring AT-rich sequences, with a stoichiometry of one E(2)-ER dimer per ERE. In contrast, highly purified E(2)-ER binds with a 10-fold lower affinity and non-cooperatively to EREs flanked by the AT-rich region. Moreover, the binding stoichiometry of highly purified E(2)-ER was 0.5 E(2)-ER dimer, or one monomer per ERE, independent of the ERE flanking sequence. Interestingly, the binding of ER liganded with the antiestrogen 4-hydroxytamoxifen (4-OHT-ER) was non-cooperative with an apparent stoichiometry of 0.5 4-OHT-ER dimer per ERE, regardless of ER purity or ERE flanking sequence. We recently showed that when 4-OHT-ER binds DNA, one molecule of 4-OHT dissociates from the dimeric 4-OHT-ER-ERE complex, accounting for the reduced apparent binding stoichiometry. In contrast, ER covalently bound by tamoxifen aziridine (TAz) gave an ERE binding stoichiometry of one TAz-ER dimer per ERE, and TAz-ER binds cooperatively to multiple AT-rich EREs, regardless of the purity of the receptor. We have obtained evidence that purification of ER removes an accessory protein(s) that interacts with ER in a sequence- and/or DNA

conformational-dependent manner, resulting in stabilization of E(2), but not 4-OHT, in the ligand binding domain when the receptor binds to DNA. We postulate that retention of ligand by ER maintains the receptor in a conformation necessary to achieve high-affinity, cooperative ERE binding. Copyright (C) 1996 Elsevier Science Ltd.

L15 ANSWER 28 OF 84 MEDLINE DUPLICATE 7
97163499 Document Number: 97163499. PubMed ID: 9010319. Triiodothyronine mimics the effects of estrogen in breast cancer cell lines. Nogueira C R; Brentani M M. (Departamento de Quimica, Disciplina de Bioquimica, Instituto de Biociencias, Universidade Estadual Paulista, Botucatu, Sao Paulo, Brazil.) JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (1996 Nov) 59 (3-4) 271-9. Journal code: AX4; 9015483. ISSN: 0960-0760. Pub. country: ENGLAND: United Kingdom. Language: English.

AB MCF-7 (estrogen receptor positive--ER+) and MDA-MB-231 (estrogen receptor negative--ER-) are human breast cancer cell lines which express functional **thyroid hormone receptors** (c-erb A alpha1 and c-erb beta1) as indicated by stimulation of mitochondrial alpha-glycerophosphate dehydrogenase. In MCF-7, mimicking E2, T3 stimulated growth in a dose-dependent (10(10) M - 10(-8) M) manner, induced the expression of progesterone receptor and growth factor TGFalpha mRNAs and inhibited that of TGFbeta mRNA; T3 also increased progesterone binding and LDH5 isozyme activities. None of these effects were observed in (ER-) MDA-MB-231 cells. 10(-6) M tamoxifen (TAM) reverted growth stimulation, suppressed progesterone receptor and TGFalpha mRNA induction and restored TGFbeta mRNA to control levels in T3-treated MCF-7 cells. That T3 is acting in MCF-7 cells via its binding to ER is suggested by the immunoprecipitation of pre-bound 125I-T3 from MCF-7 nuclear extracts by an ER-specific **monoclonal antibody** and by the displacement of 3H-estradiol binding to ER by radioinert T3.

L15 ANSWER 29 OF 84 CAPLUS COPYRIGHT 2002 ACS
1995:761770 Document No. 123:162789 Ubiquitous nuclear receptors widely distributed in animal tissues and the genes encoding them and their uses. Liao, Shutsung; Song, Ching (Arch Development Corp., USA). PCT Int. Appl. WO 9513373 A1 19950518, 196 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US12883 19941108. PRIORITY: US 1993-152003 19931110.

AB Ubiquitous nuclear receptors (UR) found in many animal tissues are characterized and cDNAs encoding them cloned for use in the manuf. of the receptor polypeptides. Methods for using the receptor polypeptides in assays designed to select substances that interact with UR polypeptides for use in diagnostic, drug design and therapeutic applications are also discussed. A cDNA library from rat vagina in .lambda.ZAPII was screened with oligonucleotides derived from the conserved DNA-binding domains of the steroid/thyroid receptor superfamily. Hybridization studies found the transcript present in ventral prostate, seminal vesicle, testis, vagina, uterus, kidney, adrenal gland, liver, spleen, brain and heart. **Antibodies** raised against a fusion protein of the UR with the trpE gene product showed that the protein was localized in the nucleus of all the tissue tested. The receptor bound to perfect direct repeats of all hormone-responsive elements tested.

L15 ANSWER 30 OF 84 CAPLUS COPYRIGHT 2002 ACS
1995:594450 Document No. 123:162780 Nucleic acids encoding mammalian H-2RIIBP or RXR.beta. (retinoid X receptor) and their uses. Ozato, Keiko (United States Dept. of Health and Human Services, USA). U.S. US 5403925 A 19950404, 14 pp. Cont.-in-part of U.S. Ser. No. 866,950, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1992-952800 19920928.

PRIORITY: US 1989-450162 19891213; US 1992-866950 19920409.

AB The present invention relates generally to the identification and characterization of new genes and proteins. More particularly, the present invention relates to the discovery of novel members of the nuclear hormone receptor superfamily and cDNA clones thereof. The family members are designated as H-2RIIBP (or RXR.beta., retinoid X receptors). These proteins bind selectively only to the native region II sequence of the conserved major histocompatibility complex class I regulatory element (MHC CRE). Sequences homologous to the H-2RIIBP gene are found in the nuclear receptor family including: retinoic acid receptors (RAR), estrogen receptors (ER), **thyroid hormone receptors** (TR), (COUP-TF), and other RXR isoformes. This invention also provides for a diagnostic test which dets. the nature and progression of a human tumor by measuring the quantity and quality of H-2RIIBP gene dosage or expression.

L15 ANSWER 31 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 8

95259440 EMBASE Document No.: 1995259440. Structure of the carboxy-terminal region of thyroid hormone nuclear receptors and its possible role in hormone-dependent intermolecular interactions. Bhat M.K.; McPhie P.; Ting Y.-T.; Zhu X.-G.; Cheng S.-Y.. Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, 37 Convent Drive,Bethesda, MD 20892-4255, United States. Biochemistry 34/33 (10591-10599) 1995.

ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.

AB The thyroid hormone nuclear receptors (TRs) are ligand-dependent transcription factors. To understand the molecular basis of ligand-dependent transactivation, we studied the structure of their carboxy-terminal activation domain. We analyzed the structures of the peptides derived from the C-terminal sequences of human TR subtypes .beta.1 (h-TR.beta.1) and .alpha.1 (h- TR.alpha.1) and a human TR mutant, PV, by circular dichroism (CD). Mutant PV has a C-terminal frameshift mutation and does not bind to the thyroid hormone, 3,3',5-triiodo-L-thyronine (T3). Analyses of the secondary structures of the peptides by CD indicate that five amino acids, EVFED, are part of an amphipathic .alpha.-helix which is required to maintain the structural integrity of the hormone binding domain. A **monoclonal antibody**, C4 (mAb C4), which recognizes both h-TR.beta.1 and h-TR.alpha.1 was developed. Using a series of truncated mutants and synthetic peptides, we mapped the epitope of mAb C4 to the conserved C-terminal amino acids, EVFED. Analysis of the binding data indicates that binding of T3 to either h-TR.beta.1 or h-TR.alpha.1 was competitively inhibited by mAb C4. Deletion of C-terminal amino acids including EVFED led to a total loss of T3 binding activity. Thus, part of the T3 binding site is located in this five amino acid segment. T3 may transduce its hormonal signal to the transcriptional machinery via interaction with EVFED at the C- terminus of TRs.

L15 ANSWER 32 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

95350937 EMBASE Document No.: 1995350937. Nuclear retinoic acid receptors in the lacrimal gland. Ubels J.L.; Dennis M.H.; Rigatti B.W.; Vergnes J.-P.; Beatty R.; Kinchington P.R.. Biology Department, Calvin College, 3201 Burton Street SE,Grand Rapids, MI 49546, United States. Current Eye Research 14/11 (1055-1062) 1995.

ISSN: 0271-3683. CODEN: CEYRDM. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB The lacrimal gland secretes and metabolizes retinoids and responds to retinoic acid in culture. Like other retinoid responsive organs it is expected to express the nuclear retinoid receptors. The goal of this study was to identify the retinoic acid receptors (RAR) in the lacrimal glands of rats, rabbits, and humans. Total RNA was prepared from whole lacrimal glands and rat lacrimal gland acinar cells grown in culture. RNA was

subjected to Northern blot analysis and probed for the RAR.alpha., RAR.beta., and RAR.gamma. mRNAs. Nuclear extracts of rat and rabbit lacrimal glands were incubated with 3H-all-trans retinoic acid and analyzed by gel filtration chromatography. Western blots of the nuclear extracts were probed using **monoclonal antibodies** to RAR.alpha. and RAR.beta.. Rat lacrimal gland expresses RAR.alpha. mRNA with two transcripts (3.8 and 3.0 kb), a single RAR.beta. mRNA transcript (3.3 kb), and a single RAR.gamma. mRNA transcript (3.3 kb). Cultured rat lacrimal acinar cells also expressed the mRNA for all three RAR subtypes. Rabbit lacrimal glands express mRNAs for RAR.alpha. (3.7 and 2.9 kb) and RAR.beta. (3.2 kb) but RAR.gamma. mRNA is not detectable. Human lacrimal glands also express mRNA for RAR.alpha. (3.5 and 2.3 kb), RAR.beta. (3.4 kb) and RAR.gamma. (3.0 kb). Lacrimal gland nuclear extracts contain proteins in the 50 kDa range that specifically bind retinoic acid with K(d) = 1.25 nM in rat lacrimal gland and 0.3 nM in rabbit. The **monoclonal antibodies** identified RAR.alpha. and RAR.beta. in both rat and rabbit lacrimal glands. The results of this study support a role for retinoids in maintaining the structure and function of the lacrimal gland. The presence of RARs suggests potential interactions of these receptors with other members of their superfamily, including androgen and thyroid receptors, which also may be involved in lacrimal function.

L15 ANSWER 33 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
95:682440 The Genuine Article (R) Number: RX180. BICISTRONIC RETROVIRAL VECTOR REVEALS CAPACITY OF V-ERBA TO INDUCE ERYTHROLEUKEMIA AND TO COOPERATE WITH V-MYB. CASINI T; GRAF T (Reprint). IST EUROPEO ONCOL, VIA RIPAMONTI 435, I-20141 MILAN, ITALY (Reprint); EUROPEAN MOLEC BIOL LAB, DIFFERENTIAT PROGRAMME, D-69012 HEIDELBERG, GERMANY. ONCOGENE (21 SEP 1995) Vol. 11, No. 6, pp. 1019-1026. ISSN: 0950-9232. Pub. country: ITALY; GERMANY. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Previous studies have shown that v-erbA and v-myb can induce the proliferation of avian erythroid cells in culture, To study the combined effects of v-erbA and v-myb, the two oncogenes were engineered into a retrovirus bicistronic vector with an internal ribosomal entry site (IRES) or into a vector with a splice acceptor (SPL), This allowed coexpression of the two proteins and a comparison with the same vector containing either v-erbA or v-myb only, Both the erbA IRES and the erbA/myb IRES virus constructs transformed erythroid cells after infection of bone marrow or blastoderm cultures. The erbA/myb IRES virus exhibited a 5-10-fold higher transformed colony forming efficiency than the erbA IRES virus in the blastoderm assay, Surprisingly, when injected into chicken embryos in the presence of helper virus, both viruses induced an erythroleukemia in about half of the animals, In contrast, no leukemia was observed with a myb IRES virus, with spliced vectors containing v-erbA alone or v-erbA in combination with v-myb nor with erbA IRES and erbA/myb IRES viruses produced in the absence of helper virus, The average latency of leukemia induction was shorter for the erbA/myb IRES virus (3.5 weeks) than for the erbA IRES virus (5 weeks), Nevertheless, for both leukemic blasts retained full factor growth, These results show that v-erbA is capable of inducing an erythroleukemia when expressed by a high titer bicistronic retrovirus under conditions of virus spreading and that its in vitro and in vivo transforming potential can be enhanced by v-myb.

L15 ANSWER 34 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
95279592 EMBASE Document No.: 1995279592. Biological and immunochemical characterization of recombinant human thyrotrophin. Canonne C.; Papandreou M.-J.; Medri G.; Verrier B.; Ronin C.. Laboratoire d'Immunochimie, Hormones Glycoproteiques, Faculte de Medecine Nord,13916 Marseille Cedex 20, France. Glycobiology 5/5 (473-481) 1995.
ISSN: 0959-6658. CODEN: GLYCE3. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Recombinant human thyroid-stimulating hormone (recTSH) has recently been engineered to detect metastatic lesions in patients operated on for thyroid cancer. In this report, we have compared the microheterogeneity, carbohydrate (CHO) content, mitogenic potency and immunoreactivity of the biotechnology product to those of human TSH of pituitary origin (pitTSH). Compositional analysis revealed that recombinant (rec) TSH produced in Chinese hamster ovary cells was overglycosylated compared with the native hormone (21 and 14%, respectively) with a higher amount of sialic acid and lack of N-acetylgalactosamine. Electrofocusing followed by immunoblotting resolved recTSH into six glycoforms with pIs ranging from 6.0 to 8.6, which were converted to a major species of pI 8.9 by sialidase treatment. pitTSH contained five main isoforms of pI 6.5-8.2 distinct from those of recTSH and partially resistant to sialidase. Binding activity of both human TSHs to porcine thyroid membrane receptors was found to be similar, but recTSH appeared to be 20% active compared to pitTSH in eliciting cAMP production and cell growth in rat FRTL-5 cells. Immunoreactivity of the recombinant hormone was investigated using polyclonal and **monoclonal antibodies** raised against the native hormone or synthetic peptide sequences of its subunits. While rec- and pitTSH were recognized to a similar extent by anti-protein **antibodies**, they exhibited a different binding pattern to antipeptide **antibodies**. Serial dilution of anti-.alpha. 1-25, anti-.alpha. 26-51, anti-.beta. 96-112 antisera bound recTSH to a greater extent than pitTSH, while anti-.beta. 31-51 and anti-.beta. 53-76 displayed similar recognition toward both preparations. Inhibition assays showed that the .alpha. 1-25 and anti-.alpha. 26-51 regions contained at least two antigenic determinants which are present in recTSD but absent in the pituitary hormone. It is therefore concluded that recTSH differs from pitTSH with respect to several conformational features at the polypeptide surface, which are likely to be responsible for altered intrinsic bioactivity and may be potentially antigenic in patients repeatedly injected with the drug.

L15 ANSWER 35 OF 84 MEDLINE DUPLICATE 9
95275298 Document Number: 95275298. PubMed ID: 7538760. Interaction of thyroid hormone nuclear receptor with **antibody**: characterization of the thyroid hormone binding site. Bhat M K; McPhie P; Cheng S Y. (Laboratory of Molecular Biology, DCBDC, National Cancer Institute, Bethesda, MD 20892, USA.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1995 May 16) 210 (2) 464-71. Journal code: 9Y8; 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB To understand the structural basis in the hormone-dependent transcriptional regulation of human beta 1 **thyroid hormone receptor** (h-TR beta 1), we characterized the region which interacted with the thyroid hormone, 3,3',5-triiodo-L-thyronine (T3). Using the hormone binding domain of h-TR beta 1 (K206-D461) as an immunogen, we screened for **monoclonal antibodies** which inhibited the binding of T3 to h-TR beta 1. mAb C3, which recognized native h-TR beta 1, was obtained. Analyses of the binding data indicate that binding of T3 to h-TR beta 1 was competitively inhibited by mAb C3. Using a series of truncated mutants of h-TR beta 1 and synthetic peptides, we mapped the binding site of mAb C3 to the region of E248-V256. Thus, part of T3 binding site in h-TR beta 1 is in this nine-amino acid segment, which was shown by circular dichroism spectroscopy to be a random coil. Based on the proposed model of the hormone binding domain as an alpha/beta barrel, E248-V256 contains part of Loop 1 which is on the same side of the DNA binding domain. These results raise the possibility that Loop 1 could be in direct contact with the nearby DNA binding domain to affect the interaction of DNA binding domain with the T3 target genes.

L15 ANSWER 36 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
95:282544 The Genuine Article (R) Number: QT366. ESTROGEN-RECEPTOR MUTANTS

WHICH DO NOT BIND 17-BETA-ESTRADIOL DIMERIZE AND BIND TO THE ESTROGEN RESPONSE ELEMENT IN-VIVO. ZHUANG Y (Reprint); KATZENELLENBOGEN B S; SHAPIRO D J. UNIV ILLINOIS, DEPT BIOCHEM, B-4 RAL, 600 S MATHEWS AVE, URBANA, IL, 61801 (Reprint); UNIV ILLINOIS, DEPT PHYSIOL & BIOPHYS, URBANA, IL, 61801. MOLECULAR ENDOCRINOLOGY (APR 1995) Vol. 9, No. 4, pp. 457-466. ISSN: 0888-8809. Pub. country: USA. Language: ENGLISH. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB To investigate the stage in estrogen receptor (ER) action at which hormone functions, we prepared human ER mutants unable to bind 17 beta-estradiol. In transfected Chinese Hamster Ovary (CHO) cells, two of the ER mutants exhibited less than 5% of the ability to activate transcription shown by wild type ER, Immunoprecipitation followed by Western blotting with **monoclonal antibodies** was used to examine the ability of the ER mutants to form heterodimers with a truncated form of wild type ER, The non-hormone-binding mutants formed heterodimers with the truncated ER as efficiently as wild type ER, We used a promoter interference assay to measure the interaction of the ER with the estrogen response element (ERE) in vivo. Expression plasmids encoding the ER mutants and wild type ER were transfected into CHO cells across a range of concentrations, resulting in both high and low levels of promoter interference, The ER mutants and wild type ER elicited similar levels of promoter interference, indicating that although they were unable to bind ligand, the ER mutants bound to the ERE in vivo as effectively as wild type ER, Additional evidence that the non-hormone-binding ER mutants are not in a functionally inactive complex comes from their ability to suppress the activity of wild type ER, when they were coexpressed in the same cells.

These data support a model for ER action in which the unliganded ER is free to dimerize and bind to the ERE, In this model, the primary role of 17 beta-estradiol in ER action is to induce a conformational change which activates the ligand-dependent transactivation domain.

L15 ANSWER 37 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 10

96162295 EMBASE Document No.: 1996162295. Mechanisms of signal transduction: Sex hormones, their receptors and clinical utility. Wittliff J.L.; Raffelsberger W.. Hormone Receptor Laboratory, James Graham Brown Cancer Center, 529 South Jackson Street,Louisville, KY 40292, United States. Journal of Clinical Ligand Assay 18/4 (211-235) 1995. ISSN: 1081-1672. CODEN: JCLAF5. Pub. Country: United States. Language: English. Summary Language: English.

AB From the first moment of development in the uterus, the human embryo is exposed to a variety of hormones. Both steroid and peptide hormones play essential roles in the development and differentiation of organs, culminating in a normal functioning individual. The human female synthesizes different sex hormones at various stages of the reproductive cycle beginning with estradiol-17.beta. formation in the ovary. In pregnancy, estriol is the principal estrogen produced by the fetal-placental unit. After menopause, estrone is synthesized from androstenedione and dehydroepiandrosterone, androgenic compounds that serve as precursors of the estrogens. When ovarian function has ceased in the postmenopausal woman, estrone is formed in peripheral organs, particularly adipose tissue under the control of the enzyme aromatase. The conjugated estrogens, secreted as glucuronides and sulfates, are also naturally occurring compounds with weak estrogenic activity. Catechol estrogens represent another class of highly active estrogenic compounds discovered in the central nervous system. Although the concentrations of the catechol estrogens are low in relation to those of ovarian estrogens, they appear to play an important role in the evolvement of sexual behavior and possibly in cancer development. Many women are also exposed to other naturally occurring estrogens such as those of equine origin (Premarin.RTM.) used in the treatment of perimenopausal symptoms. The characteristic responses occurring in hormone-target tissues such as the uterus and breast are produced as a result of estrogens and progestins

associating with their intracellular cognate receptor proteins. These receptor molecules, which bind sex hormones with high affinity and specificity, are absolute biologic prerequisites for a cell to respond to the naturally occurring hormones. If the receptor proteins are not expressed in a target cell or if their structures are severely altered, female sex hormones will be unable to produce the normal developmental responses that occur in a woman throughout her life. Sex hormone and peptide hormone receptor levels vary with the tissue of origin and its stage of differentiation as well as with a woman's age and endocrine status. Even the administration of drugs will alter receptor protein levels in the body, thereby changing a person's sensitivity to sex hormones or sex hormone mimics. Our understanding of the mechanisms of signal transduction and the absolute requirement of a receptor protein for expression of a hormone's action opened a new era of application in clinical chemistry. It is now accepted that enzyme immunoassay and ligand-binding measurements of estrogen and progestin receptors in human breast, endometrial, and ovarian carcinomas assist in predicting patient response to administrative hormone therapies such as tamoxifen (Nolvadex.RTM.) and medroxyprogesterone acetate (Provera.RTM.). Furthermore, sex hormone receptor levels in biopsies of these carcinomas are related to patient prognosis in that the presence of elevated estrogen and progestin levels indicates the likelihood of an increased disease-free interval and overall survival. Advances in technologies, such as the generation of sequence-specific **monoclonal antibodies**, synthesis of [125I] labeled ligands of high specific radioactivity, DNA-band shift assays, gene cloning, polymerase chain reactions, and cell-based bioassays using recombinant DNA approaches, have revealed details of the mechanisms of signal transduction of the regulatory proteins in the steroid hormone receptor/**thyroid hormone receptor** superfamily. The ability of the estrogen receptor to bind a variety of structurally diverse compounds can be exploited in a clinically useful way to treat cancer, and yet it can be harmful if an endocrine-disrupting agent from the environment associates with the receptor molecule. This new knowledge holds promise that other discoveries relating signal transduction defects to disease expression will improve treatment modalities before the century concludes.

L15 ANSWER 38 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

95:156448 The Genuine Article (R) Number: QH357. NUCLEAR AND CYTOPLASMIC TRIIODOTHYRONINE-BINDING SITES IN PRIMARY SENSORY NEURONS AND SCHWANN-CELLS - AUTORADIOGRAPHIC STUDY DURING DEVELOPMENT. WALTER I B; DROZ B (Reprint). UNIV LAUSANNE, FAC MED, INST HISTOL & EMBRYOL, RUE BUGNON 9, CH-1005 LAUSANNE, SWITZERLAND (Reprint); UNIV LAUSANNE, FAC MED, INST HISTOL & EMBRYOL, CH-1005 LAUSANNE, SWITZERLAND. JOURNAL OF NEUROENDOCRINOLOGY (FEB 1995) Vol. 7, No. 2, pp. 127-136. ISSN: 0953-8194. Pub. country: SWITZERLAND. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The effects of the thyroid hormones on target cells are mediated through nuclear T-3 receptors. In the peripheral nervous system, nuclear T-3 receptors were previously detected with the **monoclonal antibody** 2B3 mAb in all the primary sensory neurons throughout neuronal life and in peripheral glia at the perinatal period only (Eur. J. Neurosci. 5, 319, 1993). To determine whether these nuclear T-3 receptors correspond to functional ones able to bind T-3, cryostat sections and in vitro cell cultures of dorsal root ganglion (DRG) or sciatic nerve were incubated with 0.1 nM [(125)I]-labeled T-3, either alone to visualize the total T-3-binding sites or added with a 10(3) fold excess of unlabeled T-3 to estimate the part due to the non-specific T-3-binding. After glutaraldehyde fixation, radioautography showed that the specific T-3-binding sites were largely prevalent. The T-3-binding capacity of peripheral glia in DRG and sciatic nerve was restricted to the perinatal period in vivo and to Schwann cells cultured in vitro. In all the primary sensory neurons, specific T-3-binding sites were disclosed in foetal as

well as adult rats. The detection of the T-3-binding sites in the nucleus indicated that the nuclear T-3 receptors are functional. Moreover the concomitant presence of both T-3-binding sites and T(3)receptors a isoforms in the perikaryon of DRG neurons infers that: 1) [(125)I]-labeled T-3 can be retained on the T-3-binding 'E' domain of nascent alpha(1) isoform molecules newly-synthesized on the perikaryal ribosomes; 2) the a isoforms translocated to the nucleus are modified by posttranslational changes and finally recognized by 2B3 mAb as nuclear T-3 receptor.

In conclusion, the radioautographic visualization of the T-3-binding sites in peripheral neurons and glia confirms that the nuclear T, receptors are functional and contributes to clarify the discordant intracellular localization provided by the immunocytochemical detection of nuclear T-3 receptors and T-3 receptor alpha isoforms.

L15 ANSWER 39 OF 84 CAPLUS COPYRIGHT 2002 ACS

1994:316754 Document No. 120:316754 A cDNA for a novel member of the steroid/**thyroid hormone receptor** family.
Kroczek, Richard; Mages, Hans Werner (Germany). PCT Int. Appl. WO 9404675 A2 19940303, 33 pp. DESIGNATED STATES: W: AU, BB, BG, BR, CA, CZ, FI, HU, JP, KP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1993-EP2223 19930819. PRIORITY: EP 1992-114134 19920819.

AB A cDNA for a novel member of the steroid/thyroid receptor family known as NOT (nuclear receptor of T-cells) is cloned and expressed. The cDNA and the protein and **antibodies** to the protein are useful in the treatment and diagnosis steroid receptor-related diseases (no data). A cDNA bank from activated human T-cells was screened for transcripts strongly upregulated by cell activation and 100 distinct cDNAs obtained. This pool was then screened for transcripts that were strongly transcribed at 2 h and 24 h post-activation and 5 such clones found. Partial sequences of one of these clones showed features typical of a steroid/thyroid receptor; the cDNA was used to screen a com. human placental genomic DNA library in .lambda.FIX to obtain an 11 kb insert. In situ hybridization showed the transcript to be abundant in fibroblasts, endothelial cells in synovial membranes and a subcompartment of spleen endothelial cells. Fragments of the protein were manufd. as fusion proteins with .beta.-galactosidase for the prepn. of antisera to the receptor that were used to demonstrate that the receptor was found in the nucleus and the cytoplasm.

L15 ANSWER 40 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

94:499358 The Genuine Article (R) Number: PB149. AVIAN MYELOBLASTIC CELL-LINES TRANSFORMED BY 2 NUCLEAR ONCOPROTEINS, P135(GAG-MYB-ETS) AND P61/63(MYC) - A MODEL OF RETINOIC ACID-INDUCED DIFFERENTIATION NOT ABROGATED BY V-ERBA. ALMOUSTAFA A E; GAUTIER R; SAULE S; DIETERLENLIEVRE F; CORMIER F (Reprint). UNIV PARIS 11, INST CURIE, 15 RUE GEORGES CLEMENCEAU, F-91405 ORSAY, FRANCE (Reprint); INST EMBRYOL CELLULAIRE & MOLEC, F-94736 NOGENT SUR MARNE, FRANCE; INST PASTEUR, CNRS, EP56, F-59019 LILLE, FRANCE. CELL GROWTH & DIFFERENTIATION (AUG 1994) Vol. 5, No. 8, pp. 863-871. ISSN: 1044-9523. Pub. country: FRANCE. Language: ENGLISH. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB We previously demonstrated that the retroviral construct MHE226 transducing both the P135gag-myb-ets and p61/63myc nuclear proteins induces solid hemopoietic tumors in early chicken embryos. In the present paper, we report the characterization of two MHE226-transformed cell lines established from such hemopoietic tumors retrieved from the heart of a 13-day embryo. Cytological analysis indicated a myeloblastic phenotype. These MHE226 cell lines were positive for the MEP17 **monoclonal antibody** but were negative for the myeloblast-specific 51/2 **monoclonal antibody**. MHE226 cell lines displayed a doubling time of about 20-24 h and were maintained for at least 1 year.

Contrary to E26 myeloblastic cell lines, MHE226 cell lines were independent of chicken myelomonocytic growth factor and could be maintained in serum-free medium. MHE226 cell lines could be induced to differentiate toward the monocytic lineage by retinoic acid. Retinoic acid inhibited proliferation of MHE226 cell lines as early as day 1. After 3 days, MHE226 cells displayed cytological, enzymatic (a-naphthyl acetate esterase and chloroacetate esterase), and functional (phagocytosis) characteristics of monocytic cells. The retinoic acid-induced differentiation of MHE226 cells could not be inhibited by v-erbA. Thus, MHE226-transformed cell lines represent a novel model of cell transformation by two nuclear oncoproteins. Furthermore, they provide a model to study molecular mechanisms implicated in the monocytic differentiation program.

L15 ANSWER 41 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
94:349713 The Genuine Article (R) Number: NP119. ALL-TRANS-RETINOIC ACID AND 1-ALPHA,25-DIHYDROXYVITAMIN D-3 COOPERATE TO PROMOTE DIFFERENTIATION OF THE HUMAN PROMYELOID LEUKEMIA-CELL LINE HL-60 TO MONOCYTES. BROWN G (Reprint); BUNCE C M; ROWLANDS D C; WILLIAMS G R. UNIV BIRMINGHAM, DEPT IMMUNOL, BIRMINGHAM B15 2TT, ENGLAND (Reprint); UNIV BIRMINGHAM, DEPT PATHOL, BIRMINGHAM B15 2TT, ENGLAND; UNIV BIRMINGHAM, DEPT MED, BIRMINGHAM B15 2TT, ENGLAND. LEUKEMIA (MAY 1994) Vol. 8, No. 5, pp. 806-815. ISSN: 0887-6924. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB A basis for differentiation therapy of leukemias is provided by knowledge of agents which induce specific lineage maturation. All-trans retinoic acid (RA) induces differentiation of HL60 cells to neutrophils and is used to treat acute promyelocytic leukemia. We observed that RA did not induce neutrophil differentiation in serum-free grown HL60 cells whereas 50 nM 1 alpha,25-dihydroxyvitamin D-3 (D-3) induced maximal monocyte differentiation. Increasing RA concentrations reduced the D-3 concentration required for monocyte differentiation. Cells treated with 5 nM D-3 showed little response, but differentiated maximally with 5 nM D-3 and 10 nM RA. The D-3 analogs MC903, EB1089 and KH1060 were more potent inducers of monocyte differentiation. The extent to which analog activity was increased after cotreatment with RA was inversely related to potency. Twenty-four hour treatment with 10 nM RA primed cells for response to 5 nM D-3; the reverse sequence being ineffective. Priming with 10 nM RA, or subsequent treatment with D-3 (5 nM), did not alter expression of mRNAs encoding receptors for D-3 (VDR), RA (RAR alpha) or 9-CIS RA (RXR alpha, beta, gamma). That RA promotes both neutrophil and monocyte differentiation has implications for the use of RA and D-3 in treatment of leukemias and provides insight into mechanisms whereby RAR, VDR and RXR facilitate monocyte differentiation.

L15 ANSWER 42 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
94:114190 The Genuine Article (R) Number: MW036. ISOFORM-SPECIFIC INDUCTION OF A RETINOID-RESPONSIVE ANTIGEN AFTER BIOLISTIC TRANSFECTION OF CHIMERIC RETINOIC ACID **THYROID-HORMONE RECEPTORS** INTO A REGENERATING LIMB. PECORINO L T; LO D C; BROCKES J P (Reprint). LUDWIG INST CANC RES, 91 RIDING HOUSE ST, LONDON W1P 8BT, ENGLAND (Reprint); UNIV COLL LONDON, DEPT BIOCHEM & MOLEC BIOL, LONDON W1P 8BT, ENGLAND; LUDWIG INST CANC RES, LONDON W1P 8BT, ENGLAND. DEVELOPMENT (FEB 1994) Vol. 120, No. 2, pp. 325-333. ISSN: 0950-1991. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Retinoic acid (RA) induces secretory differentiation in the wound epidermis of a regenerating amphibian limb. We investigated the role of individual RA receptor (RAR) types in the newt wound epidermis by introducing chimaeric RA/thyroid hormone (T3) receptors (chi alpha 1 and chi delta 1) that can be activated by T3. A biolistic particle delivery system was employed to transfect cells in the wound epidermis of a regenerating limb and approximately 10% of the cells in targeted surface

areas expressed marker genes. Both chi alpha 1 and chi delta 1 were comparable in their ability to stimulate transcription of a synthetic reporter construct through a RA response element after activation with T3 in situ. This activation was also comparable to that obtained by the endogenous complement of RARs in the RA-treated, transfected wound epidermis. The RA-inducible WE3 antigen, a marker for secretory differentiation, which distinguishes the wound epidermis from normal skin (Tassava, R. A., Johnson-Wint, B. and Gross, J. 1986, J. Exp. Zool. 239, 229-240), was used to assess the functional role of chi alpha 1 and chi delta 1. Chimaeric receptors were transfected with an alkaline phosphatase marker gene, activated with T3, and the expression of both the marker and WE3 was analyzed by double-label immunofluorescence. Newt limbs transfected with chi delta 1 showed many double-labelled cells dependent on the presence of T3, whereas contralateral limbs transfected with an alkaline phosphatase marker lacking chimaeric receptor sequences did not. Limbs transfected with chi alpha 1 did not show double-labelled cells in the presence or absence of T3, whereas in an earlier study chi alpha 1, and not chi delta 1, inhibited growth. These results indicate that specific effects of RA can be mediated by particular types of RARs and demonstrate a novel approach for studying the action of RA on its target tissues.

L15 ANSWER 43 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 11
94:116925 The Genuine Article (R) Number: MV899. TRANSIENT EXPRESSION OF 3,5,3'-TRIIODOTHYRONINE NUCLEAR RECEPTORS IN RAT OLIGODENDROCYTES - IN-VIVO AND IN-VITRO IMMUNOCYTOCHEMICAL STUDIES. BESNARD F; LUO M; MIEHE M; DUSSAULT J H; PUYMIRAT J; SARLIEVE L L (Reprint). LAB NEUROBIOL MOLEC INTERACT CELLULAIRES, CNRS, CTR NEUROCHIM, UPR 416, 5 RUE BLAISE PASCAL, F-67084 STRASBOURG, FRANCE (Reprint); LAB NEUROBIOL MOLEC INTERACT CELLULAIRES, CNRS, CTR NEUROCHIM, UPR 416, F-67084 STRASBOURG, FRANCE; SYNTHELABO RECH, DEPT BIOL, BAGNEUX, FRANCE; CHUL, UNITE ONTOGENESE & GENET MOLEC, ST FOY, PQ, CANADA. JOURNAL OF NEUROSCIENCE RESEARCH (15 FEB 1994) Vol. 37, No. 3, pp. 313-323. ISSN: 0360-4012. Pub. country: FRANCE; CANADA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB It is generally accepted that the action of thyroid hormones is mediated through specific nuclear receptors. Recent studies have demonstrated the homology of the thyroid receptor with the cellular product of the oncogen v-erbA. So far, two genes have been identified and classified as alpha and beta subtypes. In this study, the expression of nuclear triiodothyronine (T-3) receptors (NT(3)Rs) was examined in secondary cultures containing 85-90% oligodendrocytes (OL) prepared from newborn rat brain primary cultures enriched in OL. These cultures, which are able to produce myelin membranes, were examined by double immunolabelling with a **monoclonal antibody** (2B3) raised against purified rat liver NT(3)Rs and with **antibodies** against two maturation markers of OL: an early marker, galactocerebroside (GC), and myelin basic protein (MBP), which is expressed later than GC. 2B3 recognized three nuclear proteins with the same molecular weights as beta 1, alpha 1, and alpha 2 subtypes with different capacities for binding T-3. In 5-day-old OL secondary cultures (25 days, total time in culture), 2B3-NT(3)R immunoreactivity was located in 77% of morphologically immature OL (GC)(+) cells, whereas only 44% of morphologically mature OL were immunoreactive. Only 35% of the MBP(+) cells co-expressed NT(3)Rs. In the corpus callosum of developing rat brain, at all ages studied from 7-60 days postnatal, the total absence of NT(3)Rs in dark OL (morphologically mature), confirmed by ultrastructural immunocytochemistry, indicates an even more dramatic decrease during maturation. Furthermore, the percentage of medium OL (less mature) stained by 2B3 is reduced by approximately half in 60- compared to 20-day-old rat brain. It is of interest to note that the in vitro observation with maturation markers mirrors the in vivo decrease of NT(3)R expression during development. It is interesting that NT(3)Rs are absent in vivo

before the critical period of active myelination. These data indicate the presence of a nuclear T-3 binding protein in the nuclei of OL at the time of myelination both in vitro and in vivo. The transient expression of these NT(3)Rs during active myelination argues in favour of a direct effect of thyroid hormones on OL. (C) 1994 Wiley-Liss, Inc.

L15 ANSWER 44 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
94:649125 The Genuine Article (R) Number: PK521. OLIGOMERIC STRUCTURES OF CYTOSOLUBLE ESTROGEN-RECEPTOR COMPLEXES AS STUDIED BY ANTIESTROGEN RECEPTOR **ANTIBODIES** AND CHEMICAL CROSS-LINKING OF INTACT-CELLS. ROSSINI G P (Reprint); CAMELLINI L. UNIV MODENA, DIPARTIMENTO SCI BIOMED, VIA CAMPI 287, I-41100 MODENA, ITALY (Reprint). JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY (SEP 1994) Vol. 50, No. 5-6, pp. 241-252. ISSN: 0960-0760. Pub. country: ITALY. Language: ENGLISH. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The structure of estrogen-receptor complexes recovered in cytosolic extracts of MCF-7 cells treated with hormone at 20 degrees was probed by chemical crosslinking of intact cells and sample analysis with four **monoclonal** anti-estrogen receptor **antibodies**. When MCF-7 cells were treated with either glutaraldehyde or dithiobis(succinimidyl propionate), cytosoluble estrogen-receptor complexes consisted of two major forms sedimenting as 4 S monomers and 8-9 S salt-resistant oligomers. By high salt sucrose density gradient centrifugation, we could observe that the four **monoclonal** anti-estrogen receptor **antibodies** bound different forms of receptor complexes from crosslinked cells. While H222 and H226 **antibodies** could interact with any form we detected, the D75 and D547 **monoclonals** could only recognize those showing sedimentation coefficients lower than 7 S. When cytosolic extracts from [S-35]-methionine-labeled cells were subjected to immunoprecipitation with H222 and D75 anti-estrogen receptor **antibodies**, electrophoretic analysis of material extracted from immunoprecipitates revealed the presence of 65 kDa estrogen receptors. If extracts were prepared from crosslinked cells, instead, two more components with estimated molecular masses of 220 and 100 kDa were specifically immunoprecipitated by the H222 **antibody**, whereas only the 100 kDa component and the estrogen receptor were found in immunoprecipitates obtained with the D75 **monoclonal**. When estrogen-receptor complexes were immunopurified from extracts prepared after cells had been crosslinked with dithiobis(succinimidyl propionate), and the oligomers were dissociated by treatment with beta-mercaptoethanol, electrophoretic analysis of our samples showed that only the 65 kDa estrogen receptor and a 50 kDa protein were selectively immunoprecipitated by anti-estrogen receptor **antibodies**. We concluded that the structures of cytosoluble estrogen-receptor complexes in MCF-7 cells treated with hormone at 2 degrees C, include oligomeric forms which contain a 50 kDa non-steroid binding protein.

L15 ANSWER 45 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
94:51237 The Genuine Article (R) Number: MU127. ADENOVIRUS E1B ONCOPROTEIN TETHERS A TRANSCRIPTIONAL REPRESSION DOMAIN TO P53. YEW P R; LIU X; BERK A J (Reprint). UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOLEC GENET, LOS ANGELES, CA, 90024 (Reprint); UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOLEC GENET, LOS ANGELES, CA, 90024; UNIV CALIF LOS ANGELES, INST MOLEC BIOL, LOS ANGELES, CA, 90024. GENES & DEVELOPMENT (JAN 1994) Vol. 8, No. 2, pp. 190-202. ISSN: 0890-9369. Pub. country: USA. Language: ENGLISH. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Many DNA tumor viruses express a protein that inhibits transcriptional activation by the tumor-suppressing transcription factor p53. We report that adenovirus E1B 55K represses p53-mediated activation by a mechanism not described previously. E1B 55K binds p53 without displacing it from its DNA-binding site. A fusion of E1B 55K to the GAL4 DNA-binding domain represses transcription from a variety of promoters with engineered upstream GAL4-binding sites. Mutations within E1B 55K that interfere with

its transforming activity and its ability to inhibit p53-mediated trans-activation also interfere with transcriptional repression by the GAL4-55K fusion. These results demonstrate that E1B 55K functions as a direct transcriptional repressor that is targeted to p53-responsive genes by binding to p53.

L15 ANSWER 46 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
94:447921 The Genuine Article (R) Number: NX177. DOMINANT-NEGATIVE MUTANTS - TOOLS FOR THE STUDY OF PROTEIN FUNCTION IN-VITRO AND IN-VIVO. SHEPPARD D (Reprint). UNIV CALIF SAN FRANCISCO, CTR OCCUPAT & ENVIRONM HLTH, CTR LUNG BIOL, CARDIOVASC RES INST, BOX 0854, SAN FRANCISCO, CA, 94143 (Reprint); UNIV CALIF SAN FRANCISCO, DEPT MED, SAN FRANCISCO, CA, 94143. AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY (JUL 1994) Vol. 11, No. 1, pp. 1-6. ISSN: 1044-1549. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Powerful new approaches for the identification and sequencing of novel cDNAs have produced a backlog of proteins seeking functions. Traditional approaches for characterizing protein function (e.g., blocking **monoclonal antibodies** and heterologous expression) have significant limitations, especially in identifying the roles specific proteins play in vivo. An alternative approach is to engineer mutations in the protein of interest that abolish its function and that also inhibit the function of simultaneously expressed wild-type protein (dominant negative mutations). This approach has wide application to the study of a number of different kinds of proteins but tends to be most effective for proteins that need to assemble into multimers to be functional. Dominant negative mutants have already provided insights into the molecular mechanisms of action of a number of protein families, including hormone receptors, oncogenes, and growth factor receptors, and have been identified as the cause of at least a few autosomal dominant diseases. Expression of dominant negative mutants under the control of highly active lung cell-specific promoters holds great promise for the study of the roles specific proteins and protein families play in lung development, health, and disease.

L15 ANSWER 47 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
93:339330 The Genuine Article (R) Number: LD538. THE MOUSE BETA-7 INTEGRIN GENE PROMOTER - TRANSCRIPTIONAL REGULATION OF THE LEUKOCYTE INTEGRINS LPAM-1 AND M290. LEUNG E; MEAD P E; YUAN Q; JIANG W M; WATSON J D; KRISSANSEN G W (Reprint). UNIV AUCKLAND, SCH MED, DEPT MOLEC MED, AUCKLAND, NEW ZEALAND. INTERNATIONAL IMMUNOLOGY (MAY 1993) Vol. 5, No. 5, pp. 551-558. ISSN: 0953-8178. Pub. country: NEW ZEALAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The leukocyte adhesion receptors M290 (alphaM290/beta7) and LPAM-1 (alpha4beta7) comprise the beta7-subfamily of integrins, which are constitutively expressed on subsets of lymphocytes populating the mouse small intestine. They are induced de novo after in vitro activation of lymphocytes and hence may serve a more general role in inflammation. In order to understand how beta7 integrins are regulated during an immune response, we isolated and characterized the promoter region of the beta7 gene. Primer extension and rapid amplification of cDNA ends identified one major transcriptional start site in a favourable context, which resembles the initiator of terminal deoxynucleotidyl transferase. Transfection assays with a luciferase reporter gene revealed that cell-specific expression in vitro was retained in a 292 bp sequence, which contained several consensus binding motifs for transcriptional factors preferentially expressed in cells of the lymphoid lineages. Multiple retinoic acid receptor sites for steroid/**thyroid hormone receptors** which typify the leukocyte cell adhesion molecule subset of integrins are present. The beta7 promoter, like its alpha4 chain partner, contains the E box core sequence CACCTG found within the muscle creatine kinase enhancer which binds MyoD in vitro. The number of

potential DNA binding sites for transcriptional factors in the beta7 promoter parallels the complex regulation of expression of M290 and LPAM-1 in inflammation and gut mucosal immunity.

L15 ANSWER 48 OF 84 MEDLINE
93371432 Document Number: 93371432. PubMed ID: 8363616. Conformational changes of human beta 1 **thyroid hormone receptor** induced by binding of 3,3',5-triiodo-L-thyronine. Bhat M K; Parkison C; McPhie P; Liang C M; Cheng S Y. (Laboratory of Molecular Biology, DCBDC, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1993 Aug 31) 195 (1) 385-92. Journal code: 9Y8; 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB To understand the structural basis in the hormone-dependent transcriptional regulation of human beta 1 **thyroid hormone receptor** (h-TR beta 1), we studied the conformational changes of h-TR beta 1 induced by binding of 3,3',5-triiodo-L-thyronine (T3). h-TR beta 1 was treated with trypsin alone or in the presence of T3, thyroid hormone response element (TRE) or T3 together with TREs. Without T3, h-TR beta 1 was completely digested by trypsin. Binding of TREs had no effect on the tryptic digestion pattern. However, T3-bound h-TR beta 1 became resistant to tryptic digestion and yielded trypsin-resistant peptide fragments with molecular weight of 28,000 and 24,000. Chymotryptic digestion also yielded a T3-protected 24 Kd peptide fragment. Using anti-h-TR beta 1 **antibodies** and amino acid sequencing, the 28 Kd fragment was identified to be Ser202-Asp456. The 24 Kd tryptic fragments were found to be Lys239-Asp456 and Phe240-Asp456. The 24 Kd chymotryptic fragment was identified to be Lys235-Asp456. The structural changes as a result of T3 binding could serve as a transducing signal to modulate the gene regulating activity of h-TR beta 1.

L15 ANSWER 49 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
93:242749 The Genuine Article (R) Number: KX508. CHANGES IN NUCLEAR 3,5,3'-TRIIODOTHYRONINE RECEPTOR EXPRESSION IN RAT DORSAL-ROOT GANGLIA AND SCIATIC-NERVE DURING DEVELOPMENT - COMPARISON WITH REGENERATION. BARAKATWALTER I (Reprint); DUC C; PUYMIRAT J. UNIV LAUSANNE, FAC MED, INST HISTOL & EMBRYOL, RUE BUGNON 9, CH-1005 LAUSANNE, SWITZERLAND (Reprint); CHU LAVAL, ONTOGENESE & GENET MOLEC LAB, QUEBEC CITY G1V 4G2, QUEBEC, CANADA. EUROPEAN JOURNAL OF NEUROSCIENCE (01 APR 1993) Vol. 5, No. 4, pp. 319-326. ISSN: 0953-816X. Pub. country: SWITZERLAND; CANADA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The action of the thyroid hormones on responsive cells in the peripheral nervous system requires the presence of nuclear triiodothyronine receptors (NT3R). These nuclear receptors, including both the alpha and beta subtypes of NT3R, were visualized by immunocytochemistry with the specific 2B3 **monoclonal antibody**. In the dorsal root ganglia (DRG) of rat embryos, NT3R immunoreactivity was first discretely revealed in a few neurons at embryonic day 14 (E14), then strongly expressed by all neurons at E17 and during the first postnatal week; all DRG neurons continued to possess clear NT3R immunostaining, which faded slightly with age. The peripheral glial cells in the DRG displayed a short-lived NT3R immunoreaction, starting at E17 and disappearing from the satellite and Schwann cells by postnatal days 3 and 7 respectively. In the developing sciatic nerve, Schwann cells also exhibited transient NT3R immunoreactivity restricted to a short period ranging from E17 to postnatal day 10; the NT3R immunostaining of the Schwann cells vanished proximodistally along the sciatic nerve, so that the Schwann cells rapidly became free of detectable NT3R immunostaining. However, after the transection or crushing of an adult sciatic nerve, the NT3R immunoreactivity reappeared in the Schwann cells adjacent to the lesion by 2 days, then along the distal segment in

which the axons were degenerating, and finally disappeared by 45 days, when the regenerating axons were allowed to re-occupy the distal segment. It is concluded that (1) NT3R expression lasts throughout the life of the DRG neurons; (2) NT3R expression by peripheral glia is restricted to the perinatal period but may be transiently reactivated in Schwann cells after a nerve injury; and (3) cell - cell interaction with axons down-regulates the expression of NT3R by Schwann cells in both growing and regenerating nerves.

L15 ANSWER 50 OF 84 CAPLUS COPYRIGHT 2002 ACS
1993:75727 Document No. 118:75727 Hepatocyte nuclear factor 4 (HNF-4) and cloning of its cDNA. Sladek, Frances M.; Zhong, Weimin; Darnell, James E., Jr. (Rockefeller University, USA). PCT Int. Appl. WO 9211365 A1 19920709, 100 pp. DESIGNATED STATES: W: AU, CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US9733 19911223. PRIORITY: US 1990-631720 19901221.
AB DNA encoding HNF-4, cells producing HNF-4, methods of inhibiting HNF-4 function, and treatment of diseases by administering ligands for HNF-4 or apoCIII are claimed. The cDNA for rat liver HNF-4 was cloned and sequenced. HNF-4 has a structure analogous to the steroid/**thyroid hormones receptors**: it contains a zinc finger domain, and a hydrophobic C-terminus with similarity to the ligand binding domain of the other receptors. Also in the C-terminus is a proline-rich region characteristic of activator domains and possible phosphorylation sites. HNF-4 binds to its recognition site as a dimer. HNF-4 mRNA is present in liver, kidney, and intestine, but not in spleen, brain, white fat, lung, or heart. The factor binds to LF-A1 sites, but does not bind significantly to ERE, TRE, or GRE sites.

L15 ANSWER 51 OF 84 CAPLUS COPYRIGHT 2002 ACS
1993:117537 Document No. 118:117537 **Thyroid hormone receptor**. Pfahl, Magnus (La Jolla Cancer Research Foundation, USA). U.S. US 5144007 A 19920901, 15 pp. Cont. of U.S. Ser. No. 266,529, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1990-604334 19901024. PRIORITY: US 1988-266529 19881103.
AB The complete amino acid sequence of human **thyroid hormone receptor**, herbA-T, as deduced by its cDNA, is presented. **Antibodies** to herbA-T are also claimed. In vitro synthesis of the protein and studies of hormone T3 binding by the receptor are described.

L15 ANSWER 52 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
92:735582 The Genuine Article (R) Number: KC844. LIGAND-DEPENDENT CONFORMATIONAL-CHANGES IN THE PROGESTERONE-RECEPTOR ARE NECESSARY FOR EVENTS THAT FOLLOW DNA-BINDING. ALLAN G F; TSAI S Y; TSAI M J; OMALLEY B W (Reprint). BAYLOR COLL MED, DEPT CELL BIOL, HOUSTON, TX, 77030 (Reprint); BAYLOR COLL MED, DEPT CELL BIOL, HOUSTON, TX, 77030. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA (15 DEC 1992) Vol. 89, No. 24, pp. 11750-11754. ISSN: 0027-8424. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Hormones and antihormones induce related, but distinct, conformational changes in the progesterone receptor [Allan, G. F., Leng, X., Tsai, S. Y., Weigel, N. L., Edwards, D. P., Tsai, M.-J. & O'Malley, B. W. (1992) J. Biol. Chem. 267, 19513-19520]. In both cases the conformational change precedes the dissociation of heat shock proteins and binding to DNA. We have now investigated the steps in hormone action which are dependent upon this conformational change. We show that in the absence of ligand, **monoclonal antibodies** directed against different regions of the progesterone receptor can induce high-affinity binding to its response element in vitro. This **antibody**-induced DNA binding is presumably facilitated by enhanced dimerization of receptor monomers. However, **antibodies** do not induce the hormone-specific

conformational change in the progesterone receptor and do not induce in vitro transcription by the receptor. In contrast, the antiprogestin ZK98299, which inhibits receptor binding to DNA, fully induces the antihormone-specific conformational change. Thus, our data imply that steroids induce a conformational change in their receptors which is necessary for events subsequent to DNA binding, most likely for transactivation.

L15 ANSWER 53 OF 84 CAPLUS COPYRIGHT 2002 ACS
1993:53519 Document No. 118:53519 DNA binding and heteromerization of the Drosophila transcription factor chorion factor 1/ultraspiracle. Christianson, Anastasia M. Khoury; King, Dennis L.; Hatzivassiliou, Evie; Casas, Jorge E.; Hallenbeck, Paul L.; Nikodem, Vera M.; Mitsialis, S. Alex; Kafatos, F. C. (Dep. Cell. Dev. Biol., Harvard Univ., Cambridge, MA, 02138, USA). Proc. Natl. Acad. Sci. U. S. A., 89(23), 11503-7 (English) 1992. CODEN: PNASA6. ISSN: 0027-8424.

AB The Drosophila chorion factor 1/ultraspiracle (CF1/USP) transcription factor, a homolog of the retinoid X receptor, is a developmentally important member of the family of nuclear (steroid) hormone receptors. Using newly developed **monoclonal antibodies** and a full-length bacterially produced protein, the in vivo DNA-binding properties of this factor and aspects of its distribution in vivo were studied in detail. During oogenesis, CF1/USP is present both in germ-line cells and in the somatic follicular epithelium. The optimal binding site of partially purified bacterially produced CF1/USP was detd. by an in vitro selection procedure and also its binding to the follicular-specific chorion s15 promoter was characterized. In vitro this bacterially produced factor is unusual in binding to a single element (half-site); simultaneous but noncoordinate binding to a second half-site is possible if these repeated elements are organized in direct orientation and spaced adequately. However, the factor interacts synergistically with several other nuclear hormone receptors: notably, it can form in vitro heteromers with mammalian thyroid and retinoic acid receptors, binding to two half-site that are organized in either direct or inverted orientation. In vivo the factor most probably functions as a heterodimer, but its partner(s) remains to be detd.

L15 ANSWER 54 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
92:552739 The Genuine Article (R) Number: JN506. COOPERATION OF PROTOSIGNALS FOR NUCLEAR ACCUMULATION OF ESTROGEN AND PROGESTERONE RECEPTORS. YLIKOMI T; BOCQUEL M T; BERRY M; GRONEMEYER H (Reprint); CHAMBON P. FAC MED STRASBOURG, INST CHIM BIOL, INSERM, U84, CNRS, GENET MOLEC EUCARYOTES LAB, 11 RUE HUMANN, F-67085 STRASBOURG, FRANCE. EMBO JOURNAL (OCT 1992) Vol. 11, No. 10, pp. 3681-3694. ISSN: 0261-4189. Pub. country: FRANCE. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Multiple proto-signals (p-NLSs) for nuclear targeting, none of which suffices on its own, cooperate in the estrogen (ER) and progesterone (PR) receptors. In the ER, an estrogen-inducible p-NLS was found in the hormone binding domain (HBD), in addition to three lysine/arginine-rich motifs resembling prototype constitutive nuclear localization signals (NLSs). The inducible and the constitutive ER p-NLSs cooperate in the presence of estrogen and hydroxy-tamoxifen, but not in the presence of ICI 164,384. In the PR, three p-NLSs, two of which are located within and directly adjacent to the second zinc ringer, cooperate with each other and a weak hormone-inducible p-NLS in the PR HBD. No 'masking' of p-NLSs by the HBD was observed for ER and PR, while the ligand-free glucocorticoid receptor HBD inhibited the activity of both homologous and heterologous NLSs. Nuclear co-translocation experiments indicated that in vivo the stability of ER and PR dimers is hormonally controlled, but that, in the absence of the cognate ligand, ER dimers are more stable than PR dimers. This is likely to account for the differential hormone requirement of ER and PR DNA binding in vitro.

L15 ANSWER 55 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
92:167420 The Genuine Article (R) Number: HH489. HEAT-SHOCK ALTERS THE COMPOSITION OF HETEROMERIC STEROID-RECEPTOR COMPLEXES AND ENHANCES RECEPTOR ACTIVITY INVIVO. EDWARDS D P (Reprint); ESTES P A; FADOK V A; BONA B J; ONATE S; NORDEEN S K; WELCH W J. UNIV COLORADO, HLTH SCI CTR, DEPT PATHOL, DENVER, CO, 80262 (Reprint); UNIV COLORADO, HLTH SCI CTR, MOLEC BIOL PROGRAM, DENVER, CO, 80262; UNIV CALIF SAN FRANCISCO, DEPT MED, SAN FRANCISCO, CA, 94143; UNIV CALIF SAN FRANCISCO, DEPT PHYSIOL, SAN FRANCISCO, CA, 94143. BIOCHEMISTRY (10 MAR 1992) Vol. 31, No. 9, pp. 2482-2491. ISSN: 0006-2960. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Under normal cellular conditions, human progesterone receptors (PR), immune-isolated from cytosols of T47D breast cancer cells, associate with two heat shock proteins (hsps), hsp 90 and hsp 70. Receptors activated by hormone binding in vivo and extracted from nuclei with 0.5 M NaCl no longer associate with hsp 90 but retain association with hsp 70. We have examined the effect of heat shock treatment of cells on hsp-receptor interactions and on receptor function. Heat shock resulted in a partial reduction in cellular levels of PR, but receptors that remained were functional for both steroid and DNA binding activities. By steady-state [S-35]Methionine labeling prior to heat shock treatment, it was determined that heat shock did not affect the composition or maintenance of preexisting cytosolic PR.hsp 90.hsp 70 complexes. By contrast, immune isolation of PR complexes from cells pulse-labeled With [S-35]methionine showed that heat shock altered the composition of newly synthesized hsps associated with PR. After heat shock, both the highly inducible form of hsp 70 (72K hsp) and a 100K hsp were bound to cytosol PR, and inducible 72K hsp remained bound with the nuclear-activated PR. Neither of these hsps were associated in detectable amounts with PR under normal cellular conditions. With respect to receptor function, heat shock treatment substantially enhanced the activity of PR in vivo as determined by measuring hormone-dependent PR-mediated transcription of a target reporter gene (MMTV-CAT) that was stably transfected into T47D cells. Heat shock treatment alone, in the absence of hormone, did not stimulate MMTV-CAT expression nor did it affect transcription from a control reporter gene, pSV2-CAT, suggesting that enhanced receptor activity was due to an effect on PR-mediated processes and not to a general effect on transcription. Induction of the heat shock response by a related chemical stress (sodium arsenite) also enhanced PR activity in vivo. Interestingly, sodium arsenite produced both a greater induction of hsp 90 and hsp 70 synthesis and a greater fold enhancement of PR-mediated gene transcription than did heat shock. This suggests that enhancement of PR activity is related not only to induction of hsp synthesis but also to the severity of the stress response. The present results provide an indication that in certain cells there may exist an interrelationship between the activation pathways by which cells respond to stress and to steroid hormones. Possible mechanisms responsible for heat shock effects on PR activity are discussed.

L15 ANSWER 56 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 12
92:523267 The Genuine Article (R) Number: JK945. IDENTIFICATION OF NUCLEAR TRIIODOTHYRONINE RECEPTORS IN THE THYMIC EPITHELIUM. VILLAVERDE D M S; DEFRESNE M P; VANNIERDOSSANTOS M A; DUSSAULT J H; BONIVER J; SAVINO W (Reprint). INST OSWALDO CRUZ, FIOCRUZ, DEPT IMMUNOL, THYMUS RES LAB, AVE BRASIL 4365 MANGUINHOS, BR-21045 RIO DE JANEIRO, BRAZIL; FED UNIV RIO DE JANEIRO, INST BIOPHYS CARLOS CHAGAS FILHO, RIO DE JANEIRO, BRAZIL; STATE UNIV LIEGE, PATHOL ANAT & CYTOPATHOL LAB, B-4000 LIEGE, BELGIUM; UNIV LAVAL, UNITE RECH ONTOGENESE & GENET MOLEC, QUEBEC CITY G1K 7P4, QUEBEC, CANADA. ENDOCRINOLOGY (SEP 1992) Vol. 131, No. 3, pp. 1313-1320. ISSN: 0013-7227. Pub. country: BRAZIL; BELGIUM; CANADA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Thymic epithelial cell physiology is known to be under neuroendocrine control. In particular, thyroid hormones modulate thymic hormone secretion

by thymic epithelial cells in vivo and in vitro, thus suggesting the existence of specific receptors for those hormones in this component of the thymic microenvironment. Yet, thyroid hormone-binding sites have previously been detected only in crude thymus fractions and lymphocytes. We, thus, decided to search for T3 receptors in the thymic epithelium, by using an antinuclear T3 receptor **monoclonal antibody**.

In situ immunohistochemical analysis of thymic frozen sections showed nuclear labeling of both lymphoid and nonlymphoid cells in the cortex and medulla. Moreover, in vitro studies using thymic epithelial cell lines and the so-called thymic nurse cells revealed a positive reaction in the chromatin, with nucleoli remaining negative. Immunoblot data clearly showed a single protein band of 57K reactive with the antinuclear T3 receptor **antibody** in murine thymus extracts as well as in the thymic epithelial cell lines. Lastly, in vitro treatment of these cells with T3 resulted in a transient, yet profound, down-modulation of the receptor.

In conclusion, our findings provide molecular evidence that the action of thyroid hormones on thymic epithelium occurs via the typical 57K nuclear T3 receptors.

L15 ANSWER 57 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

92:195761 The Genuine Article (R) Number: HK005. THE NUCLEAR ONCOGENES V-ERBA AND V-ETS COOPERATE IN THE INDUCTION OF AVIAN ERYTHROLEUKEMIA. METZ T (Reprint); GRAF T. EUROPEAN MOLEC BIOL LAB, DIFFERENTIAT PROGRAMME, POSTFACH 102209, W-6900 HEIDELBERG, GERMANY (Reprint). ONCOGENE (MAR 1992) Vol. 7, No. 3, pp. 597-605. ISSN: 0950-9232. Pub. country: GERMANY. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The nuclear oncogenes v-erbA and v-ets are known to cooperate with other viral oncogenes in the induction of avian erythroleukemia. Thus, in the case of avian erythroblastosis virus (AEV), v-erbA enhances the effect of the tyrosine kinase-encoding v-erbB oncogene by blocking the terminal differentiation of erythroid cells. In the case of E26 virus a fusion of the product from v-ets to that of the nuclear oncogene v-myb is a prerequisite for leukemogenicity. Here we show that an artificial virus carrying both v-erbA and v-ets induces a rapid, acute erythroleukemia phenotypically similar to that induced by AEV. In contrast, virus constructs containing either v-erbA or v-ets alone are non-leukemogenic, although they are capable of transforming erythroid cells in vitro. Analysis of in vitro-transformed cells showed that v-erbA induces a block of differentiation without abrogating dependence on anemic serum, while v-ets predominantly causes anemic serum independence. As expected, cells transformed by both oncogenes exhibit an increased proliferative potential, are blocked in differentiation and are anemic serum independent. These data demonstrate that two separately expressed nuclear oncoproteins can complement each other in vitro and in vivo. They also show that the v-Ets protein on its own can contribute to leukemogenesis.

L15 ANSWER 58 OF 84 MEDLINE

93010703 Document Number: 93010703. PubMed ID: 1396351. Recognition of a 56 kDa protein in partially purified rat hepatic nuclear **thyroid hormone receptor** by anti-human c-erb A beta **antibody**. Ichikawa K; Hashizume K; Kobayashi M; Nishii Y; Sakurai A; Takeda T; Suzuki S; Yamada T. (Department of Geriatrics, Endocrinology and Metabolism, Shinshu University School of Medicine, Matsumoto, Japan.) ENDOCRINOLOGIA JAPONICA, (1992 Apr) 39 (2) 203-7. Journal code: EG5; 0376546. ISSN: 0013-7219. Pub. country: Japan. Language: English.

AB Human beta **thyroid hormone receptor** (c-erb A beta protein) produced by an Escherichia coli expression system was purified by sequential column chromatography followed by electroelution from an electrophoresis gel and an **antibody** was prepared. The **antibody** recognized a 56 kDa protein band in a partially purified rat hepatic nuclear **thyroid hormone receptor**

fraction on Western blotting. Although multiple bands appeared on Western blotting of crude rat hepatic receptor preparations, a 56 kDa band was the most prominent and preadsorption of the **antibody** by purified c-erb A protein resulted in almost complete disappearance of the 56 kDa band, indicating that the 56 kDa band was formed by a specific antigen-**antibody** interaction. Furthermore, the 56 kDa protein appeared to co-elute with 3, 5, 3'-triiodo-L-thyronine binding activity in hydroxylapatite, Sephacryl S-200, and DNA-cellulose column chromatography of rat hepatic nuclear receptor, and sequential column purification resulted in selective enrichment of the 56 kDa band. These results suggest that the 56 kDa protein may be the major component of the rat hepatic **thyroid hormone receptor**.

L15 ANSWER 59 OF 84 CAPLUS COPYRIGHT 2002 ACS

1992:401447 Document No. 117:1447 The expression of nuclear 3,5,3'-triiodothyronine receptors is induced in Schwann cells by nerve transection. Barakat-Walter, I.; Duc, C.; Sarlieve, L. L.; Puymirat, J.; Dussault, J. H.; Droz, B. (Fac. Med., Univ. Lausanne, Lausanne, 1005, Switz.). Exp. Neurol., 116(2), 189-97 (English) 1992. CODEN: EXNEAC. ISSN: 0014-4886.

AB The effects of thyroid hormones on the nervous system are mediated by the presence of nuclear T3 receptors (NT3R). The expression of NT3R was investigated in spinal cord, dorsal root ganglia (DRG), or sciatic nerve of adult rats after immunostaining with a 2B3-NT3R **monoclonal antibody** which recognizes both .alpha. and .beta. types of NT3R. The 2B3-NT3R **monoclonal antibody** recognized one band corresponding to a mol. wt. of 57 kDa in ext. of spinal cord or DRG. No staining was obsd. on immunoblot of intact sciatic nerve. In the spinal cord, the nuclei of the neurons and glial cells (including both astrocytes and oligodendrocytes) exhibited 2B3-NT3R immunoreactivity. All the nuclei of the DRG sensory neurons expressed the NT3R, but all the nuclei of the satellite and Schwann cells were devoid of any immunoreaction. In the sciatic nerve, the nuclei of the Schwann cells also lacked 2B3-NT3R immunoreactivity. After sciatic nerve transection in vivo, Schwann cell nuclei, which never expressed NT3R in intact nerves of adult rats, displayed a clear 2B3-NT3R immunoreaction in proximal and distal stumps adjacent to the section. Double immunostaining with **antibodies** raised to 3-sulfogalactosylceramide or S100 confirmed that most of the NT3R-contg. nuclei belong to Schwann cells. In dissocd. cell cultures grown in vitro from sciatic nerves, Schwann cells exhibited 2B3-NT3R immunoreactivity. These data suggest that the inhibition of NT3R expression in Schwann cells ensheathing axons in intact nerve is reversed when the axons are degenerating or lacking. Thus, Schwann cells which express 2B3-NT3R in the absence of axonal contact could be responsive to T3 in regenerating peripheral nerve.

L15 ANSWER 60 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

92:413759 The Genuine Article (R) Number: JB814. PROTOONCOGENE ERBA EXPRESSION AND INCREASED ABUNDANCE OF PROGESTERONE RECEPTORS IN THE MOUSE UTERUS AFTER PASSIVE-IMMUNIZATION AGAINST PROGESTERONE BEFORE IMPLANTATION . WHYTE A (Reprint); WANG M W; CHENG J T; HEAP R B. AFRC, INST ANIM PHYSIOL & GENET RES, DEPT IMMUNOL, CAMBRIDGE RES STN, CAMBRIDGE CB2 4AT, ENGLAND (Reprint); AFRC, INST ANIM PHYSIOL & GENET RES, DEPT MOLEC PHYSIOL, CAMBRIDGE CB2 4AT, ENGLAND. JOURNAL OF REPRODUCTIVE IMMUNOLOGY (AUG 1992) Vol. 22, No. 2, pp. 153-172. ISSN: 0165-0378. Pub. country: ENGLAND. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Passive immunisation with a **monoclonal** anti-progesterone **antibody** (DB3) prevents pregnancy in the mouse, and **antibody** is localised in the endometrium before the onset of implantation. BALB/c female mice were injected intraperitoneally with 9 nmol of DB3 (a dose known to cause 100% infertility) 32 h post coitum, and the uterus was removed at various times after injection. Using a

monoclonal anti-progesterone receptor **antibody** (PR6), expression of progesterone receptors was found to be abundant in uterine tissue of DB3-treated mice; this was associated with substantial progesterone receptor mRNA levels and with maximum localisation of DB3 **antibody** as detected by anti-idiotype **antibody**. Control animals treated with an equal amount of the mouse myeloma protein P3 showed very low levels of progesterone receptor in the uterus. DB3 treatment also affected uterine expression of the proto-oncogene erbA product (which shows primary sequence homology with the progesterone receptor) as revealed by specific antiserum to the ERBA protein and by in situ hybridisation with a cDNA probe to v-erbA. Time-course studies indicated that the erbA gene was expressed at a high level before progesterone receptor expression increased, that its expression was dependent on the presence of the embryo and that erbA expression persisted longer in DB3-treated females. The observations suggest that anti-progesterone immunisation has a direct effect within the uterus, involving persistence of proto-oncogene erbA expression (which itself may represent an early maternal response to pregnancy) and increased progesterone receptor levels resulting from an unopposed oestrogen effect derived from local ligand withdrawal.

L15 ANSWER 61 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
92:517644 The Genuine Article (R) Number: JK834. A SINGLE RECEPTOR IDENTICAL WITH THAT FROM INTESTINE/T47D-CELLS MEDIATES THE ACTION OF 1,25-DIHYDROXYVITAMIN-D-3 IN HL-60-CELLS. GOTO H; CHEN K S; PRAHL J M; DELUCA H F (Reprint). UNIV WISCONSIN, COLL AGR & LIFE SCI, DEPT BIOCHEM, 420 HENRY MALL, MADISON, WI, 53706. BIOCHIMICA ET BIOPHYSICA ACTA (17 AUG 1992) Vol. 1132, No. 1, pp. 103-108. ISSN: 0006-3002. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Two anti-vitamin D receptor **monoclonal antibodies** binding to two different epitopes immunoprecipitate 100% of the HL-60 1,25-dihydroxyvitamin D-3 binding activity, while another **monoclonal antibody** specific for the porcine receptor precipitates none. Using a rat receptor cDNA probe, a single mRNA species of 4.6 kb was detected by Northern analysis of HL-60 mRNA. Using a cDNA probe from the cloned rat receptor, 10(7) recombinants from a lambda-gt11 cDNA library constructed from mRNA isolated from HL-60 cells was screened yielding two positive clones. These clones had sequences identical with the known human receptor sequence from intestinal/T47D sources. Using PCR technology, the entire sequence of the HL-60 1,25-dihydroxyvitamin D-3 receptor was determined. This sequence was found identical with that reported for the human intestinal/T47D cDNA encoding the vitamin D receptor except for a single base. The substitution of this particular base does not alter the amino acid sequence however. Thus, the same receptor likely operates in differentiation and calcium transport functions.

L15 ANSWER 62 OF 84 MEDLINE DUPLICATE 13
92388865 Document Number: 92388865. PubMed ID: 1517709. Distribution of the nuclear **thyroid-hormone receptor** in extraocular and skeletal muscles. Schmidt E D; Schmidt E D; van der Gaag R; Ganpat R; Broersma L; de Boer P A; Moorman A F; Lamers W H; Wiersinga W M; Koornneef L. (Department of Ophthalmology, University of Amsterdam, The Netherlands.) JOURNAL OF ENDOCRINOLOGY, (1992 Apr) 133 (1) 67-74. Journal code: I1J; 0375363. ISSN: 0022-0795. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The correlation between the occurrence of Graves' ophthalmopathy and Graves' hyperthyroidism may indicate a role for tri-iodothyronine (T3) hormone in the pathogenesis of Graves' ophthalmopathy. In Graves' ophthalmopathy the recti eye muscles are greatly enlarged whereas skeletal muscles seem unaffected. The distribution of the nuclear T3 receptor was studied in normal human and rat eye and skeletal muscles with

immunohistochemistry using mouse (**monoclonal**) **antibodies**
, and by in-situ hybridization for the detection of mRNA encoding the T3-receptor protein. Nuclear staining with T3-receptor **antibodies** was found in all types of tissues studied. Cytoplasmic staining occurred predominantly in the muscle fibres of the orbital layer of the eye muscles and was generally absent or very low in skeletal muscle fibres and hepatocytes. Immunostaining could be inhibited by preabsorbing the **antibodies** with bacterially expressed T3-receptor protein, implying specificity. The presence of nuclear and cytoplasmic hormone-free T3 receptor sites was indicated after preincubation of sections with T3 hormone; T3-receptor immunostaining decreased and T3-hormone staining increased. In-situ hybridization clearly revealed the presence of alpha-1 and beta-1 forms of the T3-receptor mRNA in liver, skeletal muscles, and orbital and intermediate layers of the eye muscles. The data demonstrate the presence of T3 hormone-receptor molecules in the extraocular and skeletal muscles. The different susceptibilities of these muscles to Graves' hyperthyroidism may relate to the quantitative differences in T3 hormone-receptor distribution.

L15 ANSWER 63 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
92:245679 The Genuine Article (R) Number: HM854. DISTRIBUTION OF THE NUCLEAR **THYROID-HORMONE RECEPTOR** IN EXTRAOCULAR AND SKELETAL-MUSCLES. SCHMIDT E D (Reprint); SCHMIDT E D L; VANDERGAAG R; GANPAT R; BROERSMA L; DEBOER P A J; MOORMAN A F M; LAMERS W H; WIERSINGA W M; KOORNNEEF L. UNIV AMSTERDAM, ACAD MED CTR, CTR ORBITAL, DEPT OPHTHALMOL, MEIBERGDREEF 9, 1105 AZ AMSTERDAM, NETHERLANDS (Reprint); UNIV AMSTERDAM, ACAD MED CTR, DEPT ANAT & EMBRYOL, 1105 AZ AMSTERDAM, NETHERLANDS; UNIV AMSTERDAM, ACAD MED CTR, DEPT EXPTL ENDOCRINOL, 1105 AZ AMSTERDAM, NETHERLANDS; NETHERLANDS OPHTHALM RES INST, DEPT OPHTHALMOIMMUNOL, AMSTERDAM, NETHERLANDS. JOURNAL OF ENDOCRINOLOGY (APR 1992) Vol. 133, No. 1, pp. 67. ISSN: 0022-0795. Pub. country: NETHERLANDS. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The correlation between the occurrence of Graves' ophthalmopathy and Graves' hyperthyroidism may indicate a role for tri-iodothyronine (T3) hormone in the pathogenesis of Graves' ophthalmopathy. In Graves' ophthalmopathy the recti eye muscles are greatly enlarged whereas skeletal muscles seem unaffected. The distribution of the nuclear T3 receptor was studied in normal human and rat eye and skeletal muscles with immunohistochemistry using mouse (**monoclonal**) **antibodies**
, and by in-situ hybridization for the detection of mRNA encoding the T3-receptor protein.

Nuclear staining with T3-receptor **antibodies** was found in all types of tissues studied. Cytoplasmic staining occurred predominantly in the muscle fibres of the orbital layer of the eye muscles and was generally absent or very low in skeletal muscle fibres and hepatocytes. Immunostaining could be inhibited by preabsorbing the **antibodies** with bacterially expressed T3-receptor protein, implying specificity. The presence of nuclear and cytoplasmic hormone-free T3 receptor sites was indicated after preincubation of sections with T3 hormone: T3-receptor immunostaining decreased and T3-hormone staining increased. In-situ hybridization clearly revealed the presence of alpha-1 and beta-1 forms of the T3-receptor mRNA in liver, skeletal muscles, and orbital and intermediate layers of the eye muscles.

The data demonstrate the presence of T3 hormone-receptor molecules in the extraocular and skeletal muscles. The different susceptibilities of these muscles to Graves' hyperthyroidism may relate to the quantitative differences in T3 hormone-receptor distribution.

L15 ANSWER 64 OF 84 CAPLUS COPYRIGHT 2002 ACS
1992:1748 Document No. 116:1748 Human thyroid-stimulating hormone receptor cDNA cloning and use in **monoclonal antibody** preparation. Milgrom, Edwin; Misrahi, Micheline; Loosfelt, Hugues; Atger,

IV, respectively. The physiological relevance of these CTBPs is discussed.

L15 ANSWER 67 OF 84 MEDLINE DUPLICATE 14
91242480 Document Number: 91242480. PubMed ID: 1645195. Retinoic acid receptor belongs to a subclass of nuclear receptors that do not form "docking" complexes with hsp90. Dalman F C; Sturzenbecker L J; Levin A A; Lucas D A; Perdew G H; Petkovitch M; Chambon P; Grippo J F; Pratt W B. (Department of Pharmacology, University of Michigan Medical School, Ann Arbor 48109.) BIOCHEMISTRY, (1991 Jun 4) 30 (22) 5605-8. Journal code: A0G; 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB We have recently reported that, in contrast to the glucocorticoid receptor, the **thyroid hormone receptor** does not bind to hsp90 when the receptor is translated in rabbit reticulocyte lysate [Dalman, F. C., Koenig, R. J., Perdew, G. H., Massa, E., & Pratt, W. B. (1990) J. Biol. Chem. 265, 3615-3618]. All of the steroid receptors that are known to bind hsp90 are recovered in the cytosolic fraction when hormone-free cells are ruptured in hypotonic buffer. In contrast, unliganded **thyroid hormone receptors** and retinoic acid receptors are tightly associated with nuclear components. In this paper, we translated the human estrogen receptor and the human retinoic acid receptor in reticulocyte lysate and then immunoadsorbed the [35S]methionine-labeled translation products with the 8D3 **monoclonal antibody** against hsp90. The estrogen receptor is bound to hsp90, as indicated by coimmunoadsorption, but the retinoic acid receptor is not. Translation and immunoadsorption of chimeric proteins containing the DNA binding domain of one receptor and the N-terminal and COOH-terminal segments of the other show that the DNA binding finger region of the estrogen receptor is neither necessary nor sufficient for hsp90 binding. These observations suggest that there are two classes within the steroid receptor family. In one class (e.g., glucocorticoid, mineralocorticoid, sex hormone, and dioxin receptors), the receptors bind to hsp90 and remain in some kind of inactive "docking" mode until hormone-triggered release of hsp90 occurs. In the retinoic acid/thyroid hormone class, the unligated receptors do not bind to hsp90, and the receptors appear to proceed directly to their high-affinity nuclear acceptor sites without entering the "docking" state.

L15 ANSWER 68 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 15
91189260 EMBASE Document No.: 1991189260. Intracellular distribution of the endogenous and transfected .beta. form of thyroid hormone nuclear receptor visualized by the use of domain-specific **monoclonal antibodies**. Lin K.-H.; Willingham M.C.; Liang C.-M.; Cheng S.. National Cancer Institute, Building 37,Bethesda, MD 20892, United States. Endocrinology 128/5 (2601-2609) 1991.
ISSN: 0013-7227. CODEN: ENDOAO. Pub. Country: United States. Language: English. Summary Language: English.

AB To study the regulation, tissue distribution, and subcellular localization of nuclear receptor for thyroid hormone, **monoclonal antibodies** (mAbs) against the human placental c-erbA (hTR.beta.1) protein were prepared. hTR.beta.1 was expressed in Escherichia coli and purified to apparent homogeneity. The purified hTR.beta.1 was used to produce **monoclonal antibodies**. Three hybridomas, secreting mAb J51, J52, and J53, were isolated. All of these mAbs recognized hTR.beta.1. J51 and J52 belong to the immunoglobulin G1-(.kappa.) subclass; J53 is an IgM. To evaluate cross-reactivity with other classes of c-erbAs, the three mAbs were used to immunoprecipitate the in vitro translation products of human (h) TR.alpha.1, TR.alpha.2, rat (r) TR.beta.1, TR.alpha.1, and TR.alpha.2. None of these three mAbs reacted with h- or rTR.alpha.1 and TR.alpha.2. J51 did not react with rTR.beta.1, but J52 and J53 cross-reacted with rTR.beta.1 with the same activity as hTR.beta.1. To localize the epitopes in the hTR.beta.1 molecule, [35S]methionine-labeled and truncated hTR.beta.1 containing the

hormone-binding domain E (Lys235-Asp456; Lys201-Pro414), domain D (Met169-Asp456), or the DNA-binding domain C (Glu100-Asp456) were expressed in E. coli and purified. Immunoprecipitation of the above truncated hTR.beta.1 with mAbs indicated that the epitopes for J51 and J52 were located in two different sites in the A/B domain. The epitope for J53 was located in the E domain. Using immunocytochemistry and mAb J52, the endogenous TR.beta.1 in rat pituitary GH3 cells was visualized to be exclusively present in nuclei. The transfected hTR.beta.1 in monkey COS-1 and human choriocarcinoma JEG-3 cells was recognized by both J51 and J52. Interestingly, the intracellular localization of the transfected hTR.beta.1 or rTR.beta.1 in the above two cell lines depended on the level of expression. TR.beta.1 expressed at low levels was found exclusively in nuclei. However, for high level expression of TR.beta.1, cytoplasmic localization was also detected. J53, however, failed to detect nuclear fluorescence of the endogenous and transfected TR.beta.1 in fixed cells, suggesting that its antigenic site might be occluded. Localization of the endogenous and transfected TR.beta.1 in nuclei indicated that these two receptor proteins are structurally indistinguishable. Furthermore, the findings that TR.beta.1 could be localized in the cytoplasm when receptor was overexpressed suggested finite numbers of acceptor sites for TR.beta.1 in the nucleus.

L15 ANSWER 69 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
92:162324 The Genuine Article (R) Number: HH372. EVIDENCE THAT HEAT-SHOCK PROTEIN-70 ASSOCIATED WITH PROGESTERONE RECEPTORS IS NOT INVOLVED IN RECEPTOR-DNA BINDING. ONATE S A; ESTES P A; WELCH W J; NORDEEN S K; EDWARDS D P (Reprint). UNIV COLORADO, HLTH SCI CTR, DEPT PATHOL B216, 4200 E 9TH AVE, DENVER, CO, 80262; UNIV CALIF SAN FRANCISCO, DEPT PHYSIOL, SAN FRANCISCO, CA, 94143; UNIV CALIF SAN FRANCISCO, DEPT MED, SAN FRANCISCO, CA, 94143. MOLECULAR ENDOCRINOLOGY (DEC 1991) Vol. 5, No. 12, pp. 1993-2004. ISSN: 0888-8809. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB In the absence of hormone, human progesterone receptors (PR) are recovered in the cytosolic fraction of cell lysates as a multimeric complex containing the steroid-binding polypeptide, heat shock protein-90 (hsp90), and heat shock protein-70 (hsp70). Activated forms of human PR that acquire the ability to bind to DNA are dissociated from hsp90, but retain association with hps70. The present study has examined whether associated hps70 has a function in receptor-DNA binding. When activated PR was bound to specific target DNA in a gel shift assay, no hsp70 was detectable in the PR-DNA complex, as evidenced by the failure of several **antibodies** to hsp70 to affect the mobility or the amount of complexes. To determine whether hsp70 might indirectly influence DNA-binding activity, we have examined the effect of hsp70 dissociation on PR-DNA-binding activity. Dissociation was achieved either by treatment of immunoaffinity-purified immobilized PR complexes with ATP or by the binding of PR complexes to ATP-agarose, followed by elution with high salt. Under both conditions, dissociation from hsp70 neither enhanced nor impaired the ability of PR to bind to specific DNA. These results suggest that hsp70 is not involved in PR binding to DNA, either directly by participating in DNA binding or indirectly by modulating PR-DNA-binding activity. This implies that hsp70 functions at an earlier stage in the receptor activation pathway. Consistent with the known involvement of hsp70 in stabilizing unfolded states of other target proteins, we propose that hsp70 may assist in nuclear transport of PR or in assembly-disassembly of the 8-10S multimeric complex.

L15 ANSWER 70 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
91:369294 The Genuine Article (R) Number: FU005. OVEREXPRESSION OF THE HUMAN VITAMIN-D3 RECEPTOR IN MAMMALIAN-CELLS USING RECOMBINANT ADENOVIRUS VECTORS. SMITH C L (Reprint); HAGER G L; PIKE J W; MARX S J. NIDDKD, MINERAL METAB SECT, METAB DIS BRANCH, BLDG 10, ROOM 9C101, 9000 ROCKVILLE PK, BETHESDA, MD, 20892 (Reprint); NCI, EXPTL CARCINOGENESIS LAB, HORMONE

ACT & ONCOGENESIS SECT, BETHESDA, MD, 20892; BAYLOR UNIV, DEPT PEDIAT, HOUSTON, TX, 77030; BAYLOR UNIV, DEPT CELL BIOL, HOUSTON, TX, 77030. MOLECULAR ENDOCRINOLOGY (1991) Vol. 5, No. 6, pp. 867-878. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The human vitamin D3 receptor (hVDR) cDNA was cloned into the E1 region of the adenovirus genome to generate recombinant viruses which were used to infect 293 (adenovirus-transformed human fetal kidney) cells. High salt extracts from cells infected with the recombinant viruses were subjected to immunoblot analysis using a **monoclonal antibody** to chicken VDR and were shown to contain large quantities of a protein of approximately 50 kDa with a migration identical to that of the hVDR in T47D (human mammary adenocarcinoma) cells. Scatchard analysis showed that the infected cells express approximately 100-fold more receptor than T47D cells and that this receptor binds 1,25-dihydroxyvitamin D3 with high affinity. The overexpressed hVDR also binds to DNA-cellulose and is eluted with a KCI concentration similar to that determined for fully active endogenous VDR. Nuclear extracts from cells infected with the hVDR-expressing adenoviruses contain an activity that specifically binds an oligonucleotide with sequences from the rat osteocalcin vitamin D3 response element, as determined by gel mobility shift. This interaction can be inhibited by the presence of an anti-VDR **antibody**, but not by nonspecific immunoglobulins. We conclude, therefore, that the overexpressed receptor has the ligand- and DNA-binding characteristics defined for endogenous VDR and that adenoviruses can be used to efficiently express large quantities of functional hVDR in a human cell line. Finally, a second binding activity, specific for the vitamin D response element, but distinct from the VDR, has been identified in extracts from uninfected cells.

L15 ANSWER 71 OF 84 CAPLUS COPYRIGHT 2002 ACS
1993:56559 Document No. 118:56559 Thyroid hormone binding during chick embryogenesis-characterization of an additional hepatic binding site. Bellabarda, Diego; Fortier, Suzanne; Lehoux, Jean Guy; Gagnon, Jacynthe; Giguere, Alain (Fac. Med., Univ. Sherbrooke, PQ, Can.). Prog. Thyroid Res., Proc. Int. Thyroid Conf., 10th, 857-9. Editor(s): Gordon, Amirav; Gross, Jack; Hennemann, Georg. Balkema: Rotterdam, Neth. (English) 1991. CODEN: 58JYAY.

AB It is known that triiodothyronine (T3) and thyroxine (T4) bind to high affinity receptors which are coded by different genes in the various target tissues, (1). In a study on thyroid hormone binding during chick embryogenesis, the authors obsd., using a 2-sites computer model, a second binding site for T4 with an affinity of 10-7 M and a binding capacity more than 3-fold higher than that of the known T3 receptor. In the present study, they attempted to characterize this particular T4 binding site by gel chromatog., photoaffinity labeling, and trans-blotting and immunoassay with a specific **monoclonal antibody**.

L15 ANSWER 72 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
91:20118 The Genuine Article (R) Number: EQ339. TRANSCRIPTIONAL ACTIVATION AND NUCLEAR TARGETING SIGNALS OF THE HUMAN ANDROGEN RECEPTOR. SIMENTAL J A; SAR M; LANE M V; FRENCH F S; WILSON E M (Reprint). UNIV N CAROLINA, REPROD BIOL LABS, CB 7500 MACNIDER BLDG, CHAPEL HILL, NC, 27599; UNIV N CAROLINA, DEPT PEDIAT, CHAPEL HILL, NC, 27599; UNIV N CAROLINA, DEPT CELL BIOL & ANAT, CHAPEL HILL, NC, 27599; UNIV N CAROLINA, DEPT BIOCHEM, CHAPEL HILL, NC, 27599. JOURNAL OF BIOLOGICAL CHEMISTRY (1991) Vol. 266, No. 1, pp. 510-518. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The androgen receptor (AR) is a signal-transducing protein required for sexual differentiation, development, and expression of the male phenotype. A series of human AR deletion mutants were created either by site-directed mutagenesis using restriction enzyme digestion, the polymerase chain reaction, or, for a series of unidirectional NH2-terminal deletions,

Michel (Institut National de la Sante et de la Recherche Medicale (INSERM), Fr.). PCT Int. Appl. WO 9110735 A2 19910725, 49 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (French). CODEN: PIXXD2. APPLICATION: WO 1991-FR25 19910115. PRIORITY: FR 1990-397 19900115.

AB A cDNA encoding the receptor for human TSH is cloned and expressed. The protein is used for the prepn. of **monoclonal** and polyclonal **antibodies** for use in the treatment of thyroid disorders (no data). The cDNA was cloned from a human thyroid cDNA bank in .lambda.gt10 by screening with porcine luteotropin/human chorionic gonadotropin receptor cDNA. Candidate clones were sequenced and confirmed by induction of thyroid hormone binding in COS cells. Transcription of the gene was limited to the thyroid gland. **Monoclonal antibodies** were prepd. against peptides from the hormone manufd. as fusion proteins with .beta.-galactosidase or with human ubiquitin in recombinant Escherichia coli.

L15 ANSWER 65 OF 84 CAPLUS COPYRIGHT 2002 ACS

1991:529135 Document No. 115:129135 Cloning of androgen receptor and thyroid receptor-like cDNAs. Liao, Shutsung; Chang, Chawnshang (Arch Development Corp., USA). PCT Int. Appl. WO 9107423 A1 19910530, 79 pp. DESIGNATED STATES: W: JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US6015 19901019. PRIORITY: US 1989-438775 19891117.

AB CDNAs encoding human steroid and thyroid hormone-like receptors are cloned and characterized and used as diagnostic reagents. CDNA libraries in .lambda.gt11 were screened with probes for the conserved DNA-binding region of the steroid-**thyroid hormone receptor** family. Candidates were then screened with probes specific for regions specific for other members of the family to exclude them. Clones that survived this were either "thyroid receptor-like" or "androgen receptor-like". Transcription/translation of androgen receptor-like cDNA in vitro indicated a mol. wt. of 79,000 for the protein. Competition studies confirmed that the translation product bound androgens specifically. The protein was immunopptd. with serum from prostate cancer patients. Expression for the manuf. of these proteins in prokaryotic and eukaryotic hosts and the prepn. of poly- and **monoclonal antibodies** are discussed.

L15 ANSWER 66 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

91:496246 The Genuine Article (R) Number: GD635. NOVEL COLD-SENSITIVE CYTOSOLIC 3,5,3'-TRIIODO-L-THYRONINE-BINDING PROTEINS IN HUMAN RED-BLOOD-CELL - ISOLATION AND CHARACTERIZATION. FANJUL A N; FARIAS R N (Reprint). UNIV NACL TUCUMAN, CONSEJO INVEST CIENT & TECN, INST SUPER INVEST BIOL, DEPT BIOQUIM NUTR, RA-4000 TUCUMAN, ARGENTINA; INST QUIM BIOL DR BERNABE BLOJ, RA-4000 TUCUMAN, ARGENTINA. JOURNAL OF BIOLOGICAL CHEMISTRY (1991) Vol. 266, No. 25, pp. 16415-16419. Pub. country: ARGENTINA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Four cytosolic 3,5,3'-triiodo-L-thyronine-binding proteins (CTBP) were isolated from hemoglobin-free human erythrocyte on DEAE-cellulose column by linear gradient of NaCl (0-0.4 M). CTBP I, II, and IV underwent rapid loss of their activities at low temperatures, whereas CTBP III was cold-insensitive. Reactivation of cold-inactivated CTBPs by warming was obtained at 20 and 37-degrees-C. CTBP I, II, and IV were not inhibited by thiol-blocking agents, whereas CTBP III was blocked. Scatchard analysis Of L-3,5,3'-triodothyronine binding showed a high affinity site with K(d) on the order of 10-(10) M for CTBP II and K(d) values of about 10(-9) M for CTBP I and IV and of about 10(-8) M for CTBP III. The order of affinity of iodothyronine analogues to CTBPs was similar in CTBP I, II, and IV but different in CTBP III. Chromatography on Sephacryl S-200 HR showed the elution of a single peak for each CTBP. The apparent molecular weights were about 200,000, 200,000, 25,000, and 60,000 for CTBP I, II, III, and

exonuclease III digestion. Receptor mutants were expressed in monkey kidney COS cells as truncated AR proteins between 20 and 107 kDa as revealed on immunoblots, where wild type AR was a doublet of 114 and 108 kDa. Subcellular localization by immunocytochemical staining demonstrated androgen-dependent nuclear uptake of AR from a perinuclear region of the cytoplasm. A nuclear targeting signal similar in sequence and position to the glucocorticoid receptor and homologous to the SV40 large T antigen was required for androgen-induced nuclear uptake of wild type AR. AR mutants lacking the NH2-terminal and/or steroid binding domains were constitutively nuclear with reduced transcriptional activity. Transcriptional activation by wild type AR was androgen-dependent in cotransfection studies of CV1 cells using the chloramphenicol acetyltransferase reporter gene linked to the mouse mammary tumor virus promoter. Deletion mutagenesis revealed within the NH2-terminal region a domain required for full transcriptional activity and within the steroid binding domain, an inhibitory function, deletion of which yielded a constitutively active receptor. Inhibition of wild type AR by coexpression with an inactive NH2-terminal fragment suggested competition for nuclear factors required for transcriptional regulation. These studies demonstrate a concerted interplay among the domains of the AR protein in regulating gene transcription.

L15 ANSWER 73 OF 84 MEDLINE DUPLICATE 16

91378339 Document Number: 91378339. PubMed ID: 1654791. Sites of phosphorylation and photoaffinity labeling of the 1,25-dihydroxyvitamin D3 receptor. Brown T A; DeLuca H F. (Department of Biochemistry, University of Wisconsin, Madison 53706.) ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1991 May 1) 286 (2) 466-72. Journal code: 6SK; 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB The 1,25-dihydroxyvitamin D3 receptor is a member of the steroid/**thyroid hormone receptor** gene family and is thought to act by regulating transcription of specific genes. In this report, we have used peptide mapping of porcine 1,25-dihydroxyvitamin D3 receptor to localize the sites of phosphorylation, photoaffinity labeling, and **monoclonal antibody** binding. Receptor was immunoprecipitated from [32P]orthophosphate-labeled pig kidney LLC-PK1 cells grown in the absence and presence of 1,25-dihydroxyvitamin D3. Phosphorylation of receptor was induced by 1,25-dihydroxyvitamin D3. The phosphorylated receptor was digested with Staph A V8 protease within Cleveland gels and the 32P label was found entirely in a 23-kDa fragment. Similarly, receptor that was photoaffinity labeled with 1,25-dihydroxy-[26,27-3H]vitamin D3 was subjected to peptide mapping by Cleveland gels. The primary site of photoaffinity label incorporation was in the same 23-kDa peptide. This peptide was localized to a region in the center of the receptor protein, spanning part of the previously designated hinge region and roughly one-half the proposed steroid binding domain. Because phosphorylation did not occur in the DNA binding domain, it may not be involved in the binding of receptor to DNA. The localization of phosphorylation sites to this 23-kDa peptide may suggest that phosphorylation is involved in steroid binding and/or activation of the receptor.

L15 ANSWER 74 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

91:199418 The Genuine Article (R) Number: FE674. THE VITAMIN D-RESPONSIVE ELEMENT IN THE RAT BONE GLA PROTEIN GENE IS AN IMPERFECT DIRECT REPEAT THAT COOPERATES WITH OTHER CIS-ELEMENTS IN 1,25-DIHYDROXYVITAMIN D3-MEDIATED TRANSCRIPTIONAL ACTIVATION. TERPENING C M; HAUSSLER C A; JURUTKA P W; GALLIGAN M A; KOMM B S; HAUSSLER M R (Reprint). UNIV ARIZONA, ARIZONA HLTH SCI CTR, COLL MED, DEPT BIOCHEM, TUCSON, AZ, 85724. MOLECULAR ENDOCRINOLOGY (1991) Vol. 5, No. 3, pp. 373-385. Pub. country: USA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The gene for rat bone gla protein (BGP) was isolated and 1250 basepairs

(bp), including 1100 bp of 5' flanking DNA, were placed up-stream of the human GH reporter gene. After transient transfection into the osteoblast-like rat osteosarcoma cell line ROS 17/2.8, the BGP promoter demonstrated a low level of basal activity that was increased approximately 10-fold by the addition of 10(-8) M 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3]. A single 250-bp fragment (-523 to -274) was sufficient to confer hormone inducibility upon both heterologous and homologous promoters. Deletion studies, complemented by evaluation with synthetic oligomers, enabled localization of the 1,25-(OH)2D3 response element to within 19 bp (-456 to -438), containing an element with an imperfect direct repeat [GGTGA(N4)GGACA] and homology to other steroid-responsive elements. Gel retardation assays demonstrated that partially purified chick intestinal 1,25-(OH)2D3 receptor bound specifically and with high affinity to a DNA fragment containing the putative 1,25-(OH)2D3 response element, and this binding was perturbed by **monoclonal antibodies** to the 1,25-(OH)2D3 receptor. Surprisingly, the 250-bp fragment, when linked in an antisense orientation with respect to the BGP promoter, blocked basal and hormone-dependent gene expression. However, a 246-bp fragment 5' to the 250-bp element (-1100 to -855) restored 20-fold inducibility when linked to the first fragment int he same orientation, suggesting cooperativity between at least two elements to achieve the hormonal regulation observed in this gene.

L15 ANSWER 75 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)
91:421198 The Genuine Article (R) Number: FX665. VITAMIN-D3 RECEPTORS - STRUCTURE AND FUNCTION IN TRANSCRIPTION. PIKE J W (Reprint). BAYLOR UNIV, DEPT PEDIAT, HOUSTON, TX, 77030 (Reprint); BAYLOR UNIV, DEPT CELL BIOL, HOUSTON, TX, 77030. ANNUAL REVIEW OF NUTRITION (1991) Vol. 11, pp. 189-216 . Pub. country: USA. Language: ENGLISH.

L15 ANSWER 76 OF 84 MEDLINE
92346063 Document Number: 92346063. PubMed ID: 1822365.
Immunocytochemical localization of **thyroid hormone receptors** in the adult rat brain. Puymirat J; Miehe M; Marchand R; Sarlieve L; Dussault J H. (Laboratoire d'Ontogenese et de Genetique Moleculaire, CHU Laval, Sainte-Foy, Quebec, Canada.) THYROID, (1991) 1 (2) 173-84. Journal code: BJW; 9104317. ISSN: 1050-7256. Pub. country: United States. Language: English.

AB It is generally accepted that thyroid hormones act at the genomic level through an interaction with specific nuclear receptors. Using a **monoclonal antibody** raised against the rat liver nuclear L-T3 receptor (NTR), we report here the immunocytochemical localization of T3 receptors in the adult rat brain. The strongest NTR immunoreactivity was found in the olfactory bulb, the hippocampus, the dentate gyrus, the amygdala areas, and the neocortex (layers III-VI). An intermediate NTR immunoreactivity was found in the hypothalamus, whereas the thalamus, the caudate-putamen, and the pallidum were weakly NTR-immunoreactive. In the cerebellum, a strong NTR immunoreactivity was found in the nuclei of Purkinje cells, in the internal granular layer, and in some nuclei of cells located in the molecular layer. In the brainstem, a strong NTR immunoreactivity was found in the lateral mamillary nucleus and the interstitial nucleus. A weak to moderate NTR immunoreactivity was observed in the central gray matter, while the substantia nigra and the interpeduncular nucleus were weakly stained. Furthermore, we also found NTR immunoreactivity in the nuclei of ependymocytes, epithelial cells of the choroid plexus, and cells located in the white matter. At the electron microscope level, we confirm that the immunoreactivity was not only localized in the nuclei of neurons but also in the nuclei of astrocytes and medium oligodendrocytes. This study provides new information concerning the distribution of NTR in the rat brain: (1) NTR are present not only in neurons but also in glial and ependymal cells, and (2) there is a regional and cellular heterogeneity in the distribution of NTR in the central nervous system.

L15 ANSWER 77 OF 84 MEDLINE

92346062 Document Number: 92346062. PubMed ID: 1822364. Thyroid hormone action: identification of the mitochondrial **thyroid hormone receptor** as adenine nucleotide translocase.
Sterling K. (Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, New York.) THYROID, (1991) 1 (2) 167-71. Journal code: BJW; 9104317. ISSN: 1050-7256. Pub. country: United States. Language: English.

AB A preliminary report from our laboratory suggested that the thyroid hormone triiodothyronine (T3) is bound with an association constant (Ka) approximating 2 x 10(11) M-1 by adenine nucleotide translocase (AdNT) purified from beef heart mitochondria. We now report that [125I]T3 is capable of photoaffinity labeling not only purified AdNT but also the carrier in intact beef heart mitochondria. Photoaffinity labeling in intact mitochondria was appreciably greater than that observed with purified AdNT. The covalently labeled AdNT was identified by 2-dimensional electrophoresis with pI of 10 on electrofocusing and M(r) of 31,000 on SDS gel. Identification of the covalently labeled protein as authentic AdNT was substantiated by its interaction with a specific **monoclonal antibody** preparation.

L15 ANSWER 78 OF 84 SCISEARCH COPYRIGHT 2002 ISI (R)

91:617215 The Genuine Article (R) Number: GN725. PEXPRESS - A FAMILY OF EXPRESSION VECTORS CONTAINING A SINGLE TRANSCRIPTION UNIT ACTIVE IN PROKARYOTES, EUKARYOTES AND INVITRO. FORMAN B M; SAMUELS H H (Reprint). NYU MED CTR, DEPT MED, NEW YORK, NY, 10016; NYU MED CTR, DEPT PHARMACOL, NEW YORK, NY, 10016. GENE (1991) Vol. 105, No. 1, pp. 9-15. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have constructed a family of expression vectors containing a single transcription unit that is active in Escherichia coli, eukaryotic cells, and in coupled in vitro transcription-translation systems. These vectors use the Rous sarcoma virus-long terminal repeat (RSV-LTR) as the promoter/enhancer for eukaryotic cells. In vitro transcription is made possible by inclusion of a bacteriophage T7 promoter. This same promoter is actively transcribed in E. coli that produce T7 RNA polymerase. Other features of this transcription unit include a high-efficiency eukaryotic translation start codon, a phage f1 origin of DNA replication for site-directed mutagenesis and a three-frame stop codon that facilitates C-terminal deletion mutagenesis. We term this vector family, pEXPRESS.

L15 ANSWER 79 OF 84 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

91006105 EMBASE Document No.: 1991006105. The NGFI-B protein, an inducible member of the thyroid/steroid receptor family, is rapidly modified posttranslationally. Fahrner T.J.; Carroll S.L.; Milbrandt J.. Department of Pathology, Div. of Laboratory Medicine, Washington Univ. Sch. of Med., 660 South Euclid Avenue,St. Louis, MO 63110, United States. Molecular and Cellular Biology 10/12 (6454-6459) 1990.
ISSN: 0270-7306. CODEN: MCEBD4. Pub. Country: United States. Language: English. Summary Language: English.

AB The NGFI-B gene is rapidly activated by a variety of stimuli that induce cells to differentiate or proliferate. It encodes a protein with a predicted molecular mass of .simeq.61 kDa and is a member of the thyroid/steroid hormone receptor gene family. To characterize this protein, **monoclonal antibodies** were raised against a bacterial TrpE-NGFI-B fusion protein that encompasses a large portion (Glu-410 to Leu-527) of the carboxy-terminal domain of NGFI-B. These **antibodies** detected a protein that was rapidly synthesized in response to nerve growth factor (NGF) and migrated as a broad band on sodium dodecyl sulfate-polyacrylamide gels with an apparent molecular mass that ranged from 63 to 88 kDa. Pulse-chase analysis demonstrated that NGFI-B was rapidly posttranslationally modified and was a short-lived

protein. NGFI-B was found to be a phosphorylated protein, and the multiple NGFI-B species coalesced into a single, more rapidly migrating species when treated with alkaline phosphatase. PC12 cells grown in the absence of NGF contained low levels of NGFI-B that was underphosphorylated. Epidermal growth factor, phorbol ester, and the calcium ionophore A23187 stimulated the synthesis of NGFI-B that was composed largely of underphosphorylated, rapidly migrating species. In contrast, basic fibroblast growth factor, which promotes differentiation of PC12 cells, induced the synthesis of NGFI-B species similar to those synthesized in response to NGF treatment. The underphosphorylated NGFI-B found in uninduced PC12 cells was found only in the nucleus, whereas NGFI-B in NGF-stimulated PC12 cells was present in approximately equal quantities in the cytoplasm and nucleus. Consistent with the cellular distribution observed in nonstimulated PC12 cells, the highly phosphorylated species were predominantly cytoplasmic whereas the more rapidly migrating forms were nuclear.

L15 ANSWER 80 OF 84 MEDLINE DUPLICATE 17

91015748 Document Number: 91015748. PubMed ID: 1699168. Immunocytochemical localization of thyroid hormone nuclear receptors in cultured acetylcholinesterase-positive neurons: a correlation between the presence of thyroid hormone nuclear receptors and L-tri-iodothyronine morphological effects. Garza R; Puymirat J; Dussault J H. (Unite de recherche en Ontogenese et Genetique moleculaire, Centre Hospitalier de l'Universite Laval, Quebec, Canada.) NEUROSCIENCE, (1990) 36 (2) 473-82. Journal code: NZR; 7605074. ISSN: 0306-4522. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A **monoclonal antibody** against the rat liver L-tri-iodothyronine nuclear receptor and acetylcholinesterase cytochemistry were used for the localization of thyroid hormone nuclear receptors in acetylcholinesterase-positive cell nuclei in fetal rat cerebral hemisphere neuronal cultures. After 3 days in vitro, the ratio of acetylcholinesterase-positive cells that were immunoreactive for the thyroid hormone nuclear receptor to those not stained for this receptor (74-26%, respectively) remains unchanged despite an increase in the number of acetylcholinesterase-positive cells with time (from day 3 to day 21) in culture. Furthermore, the addition of 3 X 10(-8) L-tri-iodothyronine in culture did not modify this ratio or have an effect on the number of acetylcholinesterase-positive cells, but significantly increased the neurite density in those acetylcholinesterase-positive cells that were immunoreactive for the **thyroid hormone receptor**. Conversely, no difference in the neurite densities of those acetylcholinesterase-positive cells not stained for this receptor was observed when cultured in the presence or absence of thyroid hormone. In other experiments with the same fetal brain cultures, treatment of cultures for 8 days with L-tri-iodothyronine, beginning on culture day 20, demonstrated the presence of a critical period which occurs in vitro around day 20, since the stimulatory effect of L-tri-iodothyronine on immunoreactive acetylcholinesterase-positive cell neurite density is lost after 20 days in vitro. These results demonstrate, for the first time, the presence of L-tri-iodothyronine nuclear receptors in fetal rat acetylcholinesterase-positive neurons and the existence of a cellular heterogeneity in the distribution of the **thyroid hormone receptor**. The presence of these receptors in fetal brain acetylcholinesterase-positive neurons suggests that some effects of L-tri-iodothyronine on the maturation of a subpopulation of acetylcholinesterase-positive neurons may result from a direct effect of this hormone through an interaction with its specific nuclear receptors.

L15 ANSWER 81 OF 84 MEDLINE DUPLICATE 18

90073628 Document Number: 90073628. PubMed ID: 2556118. Localization of c-ERB A proteins in rat liver using **monoclonal antibodies**. Schmidt E D; van Beeren H C; Korfage H; Dussault J H; Wiersinga W M; Lamers W H. (Department of Anatomy and Embryology, Academic

Medical Centre, Amsterdam, The Netherlands.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1989 Nov 15) 164 (3) 1053-9. Journal code: 9Y8; 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB **Monoclonal antibodies** were raised against the nuclear **thyroid hormone receptors** encoded by c-ERB A genes and against a purified nuclear receptor fraction. These **antibodies** recognize the c-ERB A protein in nuclear extracts from rat liver and are able to compete with thyroid hormone in Scatchard analyses. In sections of rat liver they react with all the hepatocyte nuclei as well as with the cells of the hepatic bile ducts. Comparison with another putative T3 receptor **antibody**, described previously, showed that distinct 57 kD proteins with a different cellular distribution were recognized.

L15 ANSWER 82 OF 84 MEDLINE DUPLICATE 19

89314401 Document Number: 89314401. PubMed ID: 2568604.

Immunocytochemical localization of thyroid hormone nuclear receptors in cultured hypothalamic dopaminergic neurons. Puymirat J; Luo M; Dussault J H. (Laboratoire d'Ontogenese et de genetique moleculaire, CHU Laval, Quebec, Canada.) NEUROSCIENCE, (1989) 30 (2) 443-9. Journal code: NZR; 7605074. ISSN: 0306-4522. Pub. country: ENGLAND: United Kingdom. Language: English.

AB By means of a **monoclonal antibody** against the rat liver L-triiodothyronine nuclear receptor and a polyclonal anti-tyrosine hydroxylase serum, it has been possible to demonstrate thyroid hormone nuclear receptors in immunoreactive tyrosine hydroxylase cell nuclei in fetal rat hypothalamic cultures. After 8 days in vitro, the ratio of tyrosine hydroxylase cells that were immunoreactive for the **thyroid hormone receptor** to those not stained for this receptor (64% to 36% respectively) remains unchanged despite an increase in the number of tyrosine hydroxylase-positive cells with time (from day 8 to day 21) in culture. The presence of thyroid hormone nuclear receptor in dopaminergic neurons is correlated with a morphological effect of L-triiodothyronine in this neuronal population. Our results demonstrate, for the first time, the presence of triiodothyronine nuclear receptors in fetal rat dopaminergic neurons and the existence of a cellular heterogeneity in the distribution of the **thyroid hormone receptor**. The presence of these receptors in fetal hypothalamic dopaminergic neurons suggests that some effects of L-triiodothyronine on the maturation of DA neurons may result from a direct effect of this hormone through an interaction with its specific nuclear receptors.

L15 ANSWER 83 OF 84 MEDLINE DUPLICATE 20

89209214 Document Number: 89209214. PubMed ID: 2539925.

Immunocytochemical localization of nuclear 3,5,3'-triiodothyronine (L-T3) receptors in astrocyte cultures. Luo M; Puymirat J; Dussault J H. (Unite de Recherche en Ontogenese et Genetique Moleculaire, Le Centre Hospitalier de l'Universite Laval, Ste-Foy, Que., Canada.) BRAIN RESEARCH. DEVELOPMENTAL BRAIN RESEARCH, (1989 Mar 1) 46 (1) 131-6. Journal code: DBR; 8908639. ISSN: 0165-3806. Pub. country: Netherlands. Language: English.

AB By means of a **monoclonal antibody** (mab) against the rat liver nuclear L-T3 receptor (NT3R) and a polyclonal anti-GFAp serum, it has been possible to demonstrate nuclear **thyroid hormone receptors** in astrocyte cultures. On day 3, 47% of GFAp+ cell nuclei were labeled by 2B3 mab. Between day 3 and day 15, the number of GFA+ cell nuclei stained by 2B3 mab increased from 47 to 75%. Thyroid hormone nuclear receptors were present in fibrous and protoplasmic astrocytes. However, they developed asynchronously in both types of astrocytes. Indeed, 60% of fibrous astrocytes were stained by 2B3 mab on day 3 and this percentage reached 77% after 8 days in vitro. In contrast, only 30% of protoplasmic astrocytes were immunoreactive for 2B3

mab on day 3 and this percentage increased slowly reaching 47% on day 8 and around 75-80% on day 15. By immunoblotting, the **monoclonal antibody** recognized two bands of proteins with a molecular weight of 57 and 45 kDa respectively. These proteins have the same electrophoretic mobility as [125I]bromoacetyl-LT3 rat liver nuclear L-T3 receptor. This paper presents the first immunocytochemical localization of nuclear L-T3 receptors in astrocyte cultures. Furthermore, we show that **thyroid hormone receptors** develop more rapidly in fibrous than in protoplasmic astrocytes.

L15 ANSWER 84 OF 84 MEDLINE

89039911 Document Number: 89039911. PubMed ID: 2903441. Differences in **antibody** recognition of the triiodothyronine nuclear receptor and c-erbA products. Freake H C; Santos A; Goldberg Y; Ghysdael J; Oppenheimer J H. (Department of Medicine, University of Minnesota, Minneapolis 55455.) MOLECULAR ENDOCRINOLOGY, (1988 Oct) 2 (10) 986-91. Journal code: NGZ; 8801431. ISSN: 0888-8809. Pub. country: United States. Language: English.

AB The in vitro translated products of several c-erbA cDNAs have recently been shown to bind thyroid hormones with high affinity and have been termed **thyroid hormone receptors**. We have used a panel of five erbA-related **antibodies** to probe the relationship between c-erbA translated products and **thyroid hormone receptors**, as conventionally measured by 125I-T3 labeling of nuclear extracts. All five **antibodies** immunoprecipitated the chick c-erbA translated products, but only one of them recognized chick liver and brain T3 receptor, as judged by acceleration of sedimentation through sucrose gradients. None of the **antibodies** reacted with rat liver and brain or human liver T3 receptors, although one **antibody** did immunoprecipitate a human c-erbA translated product. We conclude that the T3 receptor, as conventionally measured from these sources, is related but not identical to recently cloned c-erbA sequences.

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